A PALYNOLOGICAL STUDY OF SELECTED AMERICAN MEMBERS OF *OXALIS* L.

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DECLARATION

I, the undersigned, herby declare that the work contained in this assignment is my own original work and has not previously in its entirety or part been submitted at any university for a degree.

A. A Woldetinsae Date

SUMMARY

Oxalis L. has two centres of diversity, one in South-Central America and the other in southern Africa. Previous palynological studies of southern Africa *Oxalis* revealed four main pollen types, namely rugulate-reticulate, micro-rugulate-spinate, reticulate and supra-areolate (Dreyer 1996). The reticulate pollen type is further divided into 15 subtypes, out of which five have a monotypic status. The supra-areolate pollen type is divided into four subtypes based on exine structure. The reticulate pollen type is the most common pollen type within the South African members of *Oxalis*. The three remaining main pollen types display more complex exine structures and are therefore considered more derived than the reticulate pollen type.

The present study assessed the pollen of 50 American *Oxalis* species with three main objectives: 1. To observe pollen type variations among American members of *Oxalis*, 2. To compare pollen types from the two centres of diversity, and 3. To assess which centre of diversity house the palynologically more advanced species of *Oxalis*.

Two main pollen types are recorded from the present study, namely reticulate and verrucate pollen types. The reticulate pollen type could be further divided into 11 subtypes. Out of the 11 subtypes observed, nine also occur among South African members of *Oxalis*, while two types are only observed in the American members of *Oxalis*. The verrucate pollen type is found in a single American taxon and displays a more complex exine structure than the reticulate pollen type. In this study the reticulate pollen type proved to be the most common pollen type among the American members of *Oxalis*. The South African members of *Oxalis* display more complex pollen types than the American members of the genus.

OPSOMMING

Oxalis L. het twee diversiteitsentrums, een in Suid-Sentraal Amerika en die ander in suidelike Afrika. Palinologiese studies van suidelike Afrika *Oxalis*-taksa toon vier hoofstuifmeeltipes, naamlik gerimpel-netvormig, fyn-gerimpel-stekelrig, netvormig en supra-areolêr (Dreyer 1996). Die netvormige stuifmeeltipe word verder verdeel in 15 subtipes. Vyf van hierdie tipes het 'n monotipiese status. Die supra-areolêre stuifmeeltipe word verdeel in vier subtipes gebaseer op eksienstruktuur. Die netvormige stuifmeeltipe is die mees algemene stuifmeeltipe aanwesig in die Suid Afrikaanse *Oxalis*-taksa. Die drie oorblywende hoofstuifmeeltipes toon 'n meer komplekse eksienstruktuur en word as meer gevorderd as die netvormig stuilmeeltipe beskou.

In die huidige studie is stuifmeelkorrels van 50 Amerikaanse *Oxalis* spesies bestudeer met drie doelstellings in gedagte: 1. Om die variasie in die stuifmeeltipes van die Amerikaanse spesies van *Oxalis* te bestudeer, 2. Om die stuifmeeltipes van die twee diversiteitsentrums te vergelyk, en 3. Om vas te stel watter diversiteitsentrum het palinologies die meer gevorderde spesies van die genus *Oxalis*.

In die huidige studie is twee hoofstuifmeeltipes onderskei, naamlik netvormige en verrukate stuifmeeltipes. Die netvormige stuifmeeltipe is verder verdeel in 11 subtipes. Van die 11 subtipes wat onderskei is, kom nege tipes ook in die Suid Afrikaanse taksa van *Oxalis* voor, terwyl twee stuifmeeltipes slegs by die Amerikaanse soorte van Oxalis voorkom. Die verrukate stuifmeeltipe is slegs in 'n enkele Amerikaanse spesie gevind. Hierdie tipe toon 'n meer komplekse eksienstruktuur as die netvormige stuifmeeltipe. In die huidige studie het die netvormige stuifmeeltipe gebleik die mees algemene stuifmeeltipe in die Amerikaanse *Oxalis*-spesies te wees. Die Suid Afrikaanse taksa van *Oxalis* toon meer komplekse stuifmeeltipes as die Amerikaanse taksa.

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1 INTRODUCTION

1.1 Family Oxalidaceae

Members of the Oxalidaceae are herbs (usually with bulbs), shrubs or trees with considerable levels of soluble and crystalline oxalates in their leaves. The leaves are alternate, entire, often emarginated and lack stipules. Flowers are either solitary, axilary or borne in determinate, often umbel-like inflorescences. Each flower has five distinct sepals, five petals (distinct or basely slightly connate), usually ten fertile stamens and five carpels, forming a superior 5-lobed ovary with axile placentation. Fruits are loculicidal capsules or berries. Oxalidaceae is distributed in the tropical and sub-tropical regions of Asia, Africa and central and South-America (Dreyer 1996)

The family Oxalidaceae was originally placed in the order Geraniales (Cronquist 1981), but there has been major disagreements regarding the other families to be included in this order. Cronquist (1981) included the Geraniaceae, Limnanthaceae, Tropaeolaceae and Balsaminaceae in the Geraniales, whereas Taktajan (1980) included the Geraniaceae, Lepidobotryaceae, Hypseocharitaceae and Biebersteiniaceae.

Recently the Angiosperm Phylogenetic Group (1998) published an updated phylogeny for the angiosperms based on both morphological and molecular data (including sequence data of the rRNA, *rbcL* and *atpB* genes) and proposed a new classification system for the angiosperms. This new classification placed the Oxalidaceae in the order Oxalidales, next to the orders Malpighiales and Celastrales. These two orders were previously regarded as only distantly related to the Geraniales (Cronquist 1981).

1.1.1 Included Genera

The genera to be included in the family Oxalidaceae have been equally controversial. Knuth (1930) included *Biophytum* DC., *Oxalis*, *Averrhoa* L., *Sarcotheca* Blume, *Dapania* Korth, *Lepidobotrys* Engl., *Hypseocharis* Remy and *Eichleria* Progal in the family. Boesewinkel (1985) agreed with this list, excluding only *Eichleria*. Hutchinson (1959) placed *Averrhoa* in the Averrhoaceae within the Rutales, while *Dapania* and

Sarcotheca were transferred to the Lepidobotryaceae in the Malpigiales. Even though the genera to be included in the Oxalidaceae are still controversial, most taxonomists (Knuth 1930; Hutchinson 1959; Veldkamp 1971; Robertson 1975; Chant 1978, 1993; Cronquist 1981 and Boeswinkel 1985) agree that *Biophytum*, *Oxalis*, *Averrhoa*, *Dapania*, *Lepidobotrys* and *Hypseocharis* belong to the Oxalidaceae. However, in a study of the phylogenetic relationships of the Geraniaceae based on *rbc*L sequence data, Price and Palmer (1993) indicated that *Hypseocharis* is a close relative of the monophyletic Geraniaceae, and thus suggested the exclusion of *Hypseocharis* from the Oxalidaceae.

1.1.2 Genus *Oxalis*

The genus *Oxalis* was described by Linnaeus (1753) and named from the Greek word for acid and salt. *Oxalis* is the largest genus in the family Oxalidaceae, and is represented by *ca.* 800 species world-wide (Judd *et* al. 1999). *Oxalis* displays two centers of diversity, one in South-Central America and the other in southern Africa, where it also forms a prominent element of the Cape Flora.

The American members of *Oxalis* include *ca.* 581 species grouped into 28 sections (Huynh 1969a) and the South African members of the genus are represented by *ca.* 270 taxa belonging to 11 sections (Salter 1944).The African center of diversity is most specious in the Western Cape region of South Africa and the species are abundant within both the Fynbos and Succulent Karoo Biomes (Oberlander *et al.* 2002)

There are only a few published systematic studies for both southern African and American members of *Oxalis*. Knuth (1930) has contributed by completing alpha-taxonomic studies of both South African and American species. Salter (1944) contributed considerably to the macro-morphological and taxonomic understanding of the South African members of the family. Huynh (1969 a and b) completed palynological work on both American and South African members, but restricted her studies to light microscopic work. Lourteig (1994) delimited three subgenera, *Oxalis*, *Thamnoxys* and *Monoxalis* within the genus *Oxalis*, However, the information regarding these delimitations is not easily accessible, and thus the classification system by Knuth (1930) is followed in this study. Dreyer (1996) studied the palynology of all the South African members of the genus in detail. A molecular phylogeny based on the

*trn*L-F chloroplast region of the South African members of *Oxalis* is currently in progress (Oberlander, *pers. com*).

1.2 Terminology

Both Huynh (1969 a & b) and Dreyer (1996) used alphabetical letters A, B, C and D to name their pollen types. This becomes confusing, since one letter is used to identify two different pollen types in these studies. Therefore in the present study the letter h will appear in front of Huynh's (1969 a & b) pollen types and subtypes and the letter d in front of pollen types and subtypes described by Dreyer (1996). For example, hA will be Huynh's (1969 a & b) pollen type A while dA will be Dreyer's (1996) pollen type A. Pollen types described in the present study are presented in bold typescript.

Definitions for some of the palynological terms used in this document are given in the glossary. This terminology was based on the proposals by Punt *et al.* (1994) and Moore *et al.* (1991).

The different layers of the exine are described in two ways proposed by two different schools of thought . Erdtman (1952) distinguished a sexine and nexine layer in the exine based on a morphological criteria, while Faegeri (1956) differentiated between ectexine and endexine layers of the exine based on their staining properties (Fig 1.1). Both systems are referred to in this study. These descriptions are illustrated in Figure 1.1, which is a diagrammatic representation of a section through the exine of a pollen grain.

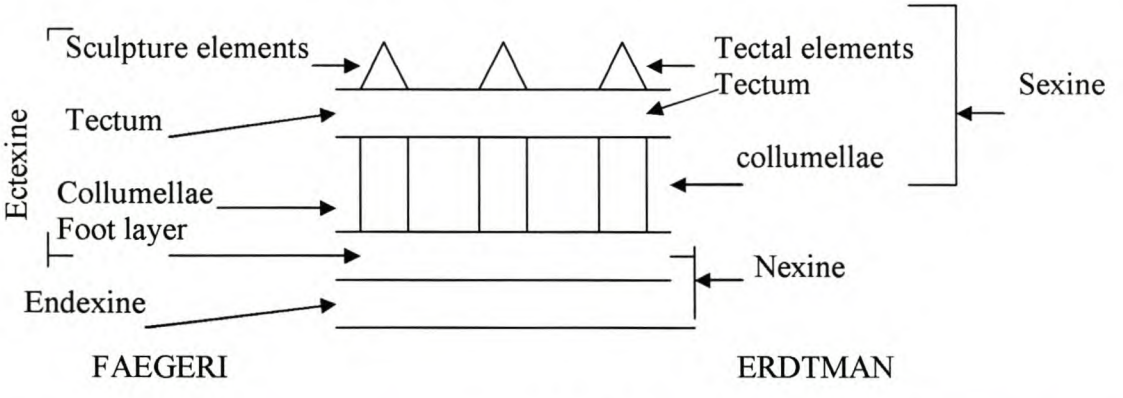


Figure 1.1 Diagrammatic representation of the different exine layers of a pollen grain wall (following Punt *et. al.* 1994).

1.3 Palynology

Palynology is the study of pollen grains and spores. It involves a study of both the structure and the formation of pollen grains and spores, their dispersal and their preservation under certain environmental conditions (Moore *et al.* 1991). Palynology is applicable to a range of scientific studies, including taxonomy (Moore *et al.* 1991).

Significant attempts to describe variations in pollen grain size and shape and to relate these to plant classification started as early as the 1830's (Stuessy 1994). Erdtman (1952) mentioned Hyde and Williams in (Hyde 1944) as the first to use the term palynology to refer to pollen and spore science. The extensive use of palynology in most systematic and evolutionary studies became possible through two major developments. The first was the development of the acetolysis method by Erdtman (1952) and the second the invention of the scanning electron microscope (SEM).

The significance of palynological data in solving taxonomic problems has been proven by many previous studies. Pollen type variation within taxonomic groups may differ from group to group. Some taxa may have uniform pollen types and others may show extreme variation (Stuessy 1994). The phylogenetic significance of palynology was further emphasized by the work of Chase *et al.* (1993). They studied the *rbc*L gene of the chloroplast genome of taxa representing all major taxonomic groups of the angiosperms, and produced a phylogenetic reconstruction for this group. Their analysis revealed that the major division in angiosperms is not a monocot versus dicots split, but rather one that correlates with pollen apertures, namely uni-aperturate versus tri-aperturate.

Although palynology has proven to be of great value in solving taxonomic problems, palynological results should always be interpreted with caution. Muller (1979) argues that the exine structure is a compromise between the protective (harmomegathic) and reservoir functions it fulfills. Therefore the exine structure is the net product of various selective pressures. This must have led to widespread parallelism and convergence in pollen grain features.

Pollen grain size is usually used as an additional feature in describing specific pollen types. However, its use in palynological classification is complicated by the fact that

stylar configurations may vary between plants of the same species in a given population (Ornduff 1970). Huynh (1969 a) also found differences in grain size in anthers from the different stamen whorls of *Oxalis* flowers. Grains from anthers of the longest staminal whorl are the largest, whereas grains from anthers of the other two whorls are progressively smaller (Hayes 2000; Trognitz *et al.* 2000).

Another problem is that the ploidy level is directly proportional to pollen grain size (Muller 1979). Polyploid plants produce larger pollen grains than diploid plants of the same species. Therefore one cannot use grain size without considering the ploidy level. Since karyological information on *Oxalis* is limited, it becomes difficult to use grain size in palynological classification on its own.

Grain shape can be useful for identification purposes. An example of this is the distinction of the African members of Lauraceae from members of the family in other parts of the world (Van der Merwe *et al.* 1990). Pollen grains of the African members of Lauraceae genus *Cryptocarya* are peroblate / oblate when rehydrated (mounted in glycerine jelly) and disc shaped oblate when dry, whereas those from Taiwan and South America have been described as spheroidal (Wang 1969; Raj & Van der Werff 1988). Shape can, however, vary within one grain type or within a particular species (Moore & Webb 1987). Additionally variation in shape can be caused by extraction methods and embedding media (Moore *et al.* 1991).

1.3.1 Palynological work in *Oxalis*

Two comprehensive palynological studies of *Oxalis* have, however, been completed by I.) Huynh (1969 a & b) and II.) Dreyer (1996), respectively

I. Huynh (1969 a & b):

The first comprehensive palynological study on the Oxalidaceae and some related families was done by Huynh (1969 a & b). This study was conducted with the aid of a light microscope (LM) and concentrated on the genus *Oxalis*, including *ca.* 420 species from both the American and African centers of diversity. She followed the classification proposed by Knuth (1930) for the American species, and that of Salter (1944) for the southern African taxa. In this comprehensive study, representatives from

all sections of the American taxa and from all endemic sections of the South African taxa were included.

Pollen grain size

Huynh (1969 a) observed a progressive grain size change from the supposedly primitive genera (*Averrhoa* and *Sarcotheca*) to the advanced genus *Oxalis* within the Oxalidaceae. She also found grain size differences between the different stylar whorls, with the longest staminal whorl producing the largest grains.

Pollen grain shape

Huynh (1969 a) referred to *Oxalis* pollen grains as being prolate.

Exine structure

Huynh (1969 a & b) used LM to study pollen grains, which limited the number of characters that could be observed from the exine. She regarded the exine as comprising of a supra-reticulum, a tectum, an infra-reticulum, a foot layer of endexine and finally an intine layer. She identified four pollen types and 13 pollen subtypes based on the presence or absence of a distinct supra-reticulum and the simple or complex constitution of apertures (colpate, porate or colporate).

Group hA: Grains are tricolpate including a few 4-5 colpate and / or 6-pantocolpate grains. A supra-reticulum is clearly visible in this group, and the group is further divided in to four subtypes. Subtypes hA1 and hA4 frequently occur within American and South African members of the genus. Subtype hA2 is only recorded from three American sections namely *Acetocella*, *Hesperoxalis* and *Neocaledoniae*. Subtype hA3 is recorded from a number of different sections in both American and South African members.

Group hB: This pollen type is similar to group hA, except that the apertures are pores. This type of pollen is observed in a single American species, *O. vulcanicola*.

Group hC: Pollen grains are tri-, tetra-, or panto-colpate and a supra-reticulum is absent or vague. Pollen types hC1 – hC3 and hC5 are described as being rare and

special, restricted to a few South African taxa that belong to section *Sagitattae*. Pollen type hC4 is also limited to a few South African taxa of the section *Angustatae*, subsections *Pardalis* and *Linearis*. Type hC6 is unique, and is recorded from a single species, *O. magellanica*, from South America and Australia.

Group hD: Pollen grains are tricolporate with a very distinct reticulum. This group is divided into two principal types, namely hD1 and hD2. They are fairly similar in structure and unique to species from South and Central America (including New Mexico).

Huyhn (1969 a & b) proposed a hypothesis that pollen grains undergo a gradual destruction (reduction) of their tectum and noted the tendency of tectum reduction in both African and American members of *Oxalis*. She speculated that the most common pollen type hA1 (dC) gave rise to hA2 through the multiplication of "network parts", and to hC6 by the destruction of the existing reticulum in American taxa. She regards hC1, hC2 and hC3 (different forms of dA) present in African taxa, as transitional stages in the process of tectum reduction. Tectum reduction continues in subtupe hC4 (dD) and the tectum is completely lost in hC5 (dB) (Huynh 1969 a &b).

Apertures

Huynh (1969a) found colporate grains to be common in the four most primitive American sections while the more advanced American sections mostly have colpate grains. Porate pollen grains were found in a single American species. She found the South African species to have colpate pollen grains only. She emphasized the taxonomic significance of apertures in the Oxalidaceae based on the presence of colporate grains in the genera *Averrhoa*, *Sarcotheca*, *Eichleria*, *Dapania*, *Hypseocharis* and *Biophytum*. These genera are generally considered to display less derived characters than *Oxalis* (Huynh 1969 a & b). She thus suggested an evolutionary change from primitive colporate grains in other genera in the Oxalidaceae to derived colpate grains in *Oxalis*.

II. Dreyer (1996):

Dreyer (1996) completed a detailed palynological study of all the southern African members of *Oxalis*. A total of 570 specimens representing 270 taxa were studied. She used LM, SEM and TEM techniques in her investigations. SEM and TEM techniques were used to study pollen wall sculpture and structure respectively. Pollen wall sculpture was primarily used to define 21 different pollen types or subtypes, while aperture number and arrangements were considered of secondary importance.

Pollen grain size

Dreyer (1996) examined pollen grains from the different stylar whorls and took polar and equatorial measurements. She found the apolar grains (type dD4) of *O. hirta* var. *intermedia* Salter (section *Angustatae*) to be the largest with an average grain diameter of 81.99 µm. The second largest grains had an average diameter of 30 – 50 X 35 – 55 µm, while the smallest grains in *O. camelopardalis* Salter (section *Angustatae*) had an average diameter of 25.26 X 26.08 µm. Generally pollen grains of the types dA, dB and dC were found to be smaller than those of type dD, supporting her delimitation based on sexine structure.

Pollen grain shape

Dreyer (1996) found pollen grain shape to be of minor importance among the southern Africa members of *Oxalis*. Most grains are triangularly rounded in polar view and spherical to oblate (seldomly prolate) in equatorial view. Apolar grains (pollen grains without distinct polarity) with a rugulate-reticulate tectum and those with a supra-areolate tectum are mostly isodiametrical, whereas some of the polar grains with a supra-aereolate tectum are almost square in polar view (Dreyer 1996).

Pollen grain exine structure

Dreyer (1996) mainly used SEM and TEM techniques for her detailed exine studies. This highlighted many more taxonomically significant characters than the LM techniques employed by Huynh (1969 a & b) could. Characters such as exine thickness, the ratio between sexine and nexine diameters, the occurrence and type of tectum, the occurrence and type of supratectal structures, the structure of the infra-reticulum, the presence or absence of a footlayer and footlayer thickness were found to

be taxonomically informative (Hong & Hedberg 1990; Luegmayr 1993). Based on the exine characters, Dreyer (1996) identified four main pollen types and 19 subtypes. Brief descriptions of these pollen types and subtypes are provided below.

Pollen type dA: Rugulate-reticulate pollen: Pollen grains with a rugulate-reticulate tectum with small, sharp supratectal spinules. Pollen grains display very little tectal variation, and grains are pantocolpate or occasionally tricolpate / tetracolpate.

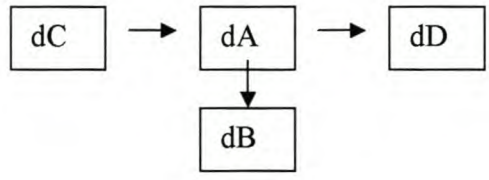
Pollen type dB: Micro-rugulate-spinate pollen: Pollen grains with a distinct micro-rugulate tectum with large supratectal spines. The grains are always tricolpate. This pollen type is observed in one South African species only, and is regarded as the evolutionary derivative of pollen type dA.

Pollen type dC: Reticulate pollen: Pollen grains semitectate with a reticulate wall structure. The grains are predominantly tricolpate, with a limited occurrence of tetracolpate or aberrant grains. Considerable structural variation exists within this pollen type; therefore detailed exine characters were used to subdivide pollen type dC into 15 subtypes. The different subtypes, along with short descriptions of each, are summarized in the cluster diagram (Fig. 1.2), following Dreyer (1996).

Pollen type dD: Supra-areolate pollen: Pollen grains have a unique supra-areolate wall structure. They are characterized by high, freestanding supratectal areaolae on an uneven, sometimes verrucate to baculate tectum traversed by micro-channels. Pollen grains are tri-, tetra- or pantocolpate. Based on variations in shape, size and arrangement of areolae, four subtypes were identified within this pollen type.

Dreyer (1996) acknowledged the same palynological variations used by Huynh (1969 a), but used different descriptions and interpretations of the pollen types (Table 3.2).

Dreyer (1996) rejected the hypothesis by Huynh (1969 a & b) of a gradual tectum destruction (or reduction) and proposed a hypothesis of gradual increase in tectum complexity. Dreyer (1996) proposed different paths of pollen grain change, and the most probable evolutionary line is schematically summarized as:



Apertures

According to Dreyer (1996), all South African members of *Oxalis* have tricolpate, tetracolpate or pantocolpate pollen grains. In rugulate-reticulate pollen grains (dA), apolar, pantocolpate grains are dominant, with only two taxa displaying tri- or tetracolpate grains. The micro-rugulate-spinate and reticulate pollen types (dB and dC respectively) are usually tricolpate, with apertural aberrations sometimes present in the reticulate pollen types. Supra-areolate pollen grains (dD) were predominantly found to be pantocolpate, although tricolpate grains were found in a few taxa. This type of pollen grain is also known for the high occurrence of apertural aberrations, a character that may suggest evolutionary instability.

1.4 Possible relationship between function and morphology of pollen grains

Generally morphology and function are interdependent. The morphology of a plant part can dictate its function and reversibly a particular morphology could be obtained through the process of adaptation to fulfill a certain function. Understanding the possible link between morphology and function of pollen grains (any plant part under study) could have a potential taxonomic relevance, since this relationship will reflect on the evolutionary history of the plant in question.

Size, shape, surface sculpturing and aperture number and structure can be considered as the basic observable morphological features of pollen grains. Angiosperm pollen grains display a large diversity of apertures and surface sculpturing, and the diversity observed between taxa is usually taxonomically significant (Punt 1986). Apart from the use of variations in the sculpture for identification purposes, their functional significance is still controversial (Moore *et al.* 1991). A number of theories regarding the function of spines, pores, grooves and the reticulation of the exine are available, and a number of papers dealing with these matters have been collected and edited by Blackmore and Ferguson (1986). The endeavor to link morphological features of the exine to specific

functions is not an easy one, since each feature may have several functions (Moore *et al.* 1991).

The mechanical structure of the exine is affected by the harmomegathic function of the pollen wall (Blackmore and Barnes 1985). Water gain or loss may also change the width of the colpi; the configuration of the elastic pore membranes (allowing changes in cytoplasmic volume) and the entire shape of the pollen grain may change with hydration, often involving an extension of the polar axis (Moore *et al.* 1991).

The known function of apertures is to allow the passage of the germinating pollen tube; therefore more than one aperture in a grain presents a better opportunity for a pollen tube to emerge close to the stigmatic surface. Studies illustrate that there is a tradeoff between pollen grain viability and rate of germination. Studies in pansies (*Viola diversifolia*) show that pollen grains with higher aperture numbers germinate quicker, but die faster than pollen with lower aperture numbers (Till-Bottraud *et al.* 1999). Till-Bottraud *et al.* (1999) speculated that hetromorphism could be favored when pollination is unpredictable, because producing both competitive (pollen grains with high aperture numbers) and long lived pollen grains (with lower aperture numbers) would give the plant a better chance of survival. Heteromorphism is also observed from certain *Oxalis* taxa, and Dreyer & Till-Bottraud (in prep.) are currently assessing the evolutionary significance of this polymorphism among selected members of southern African *Oxalis* (Dreyer, *pers. com.*). Dreyer (1996) found pollen grains of the pollen type dC usually to be tricolpate, although some apertual aberrations were found in a small percentage of grains of a few species.

Thanikaimoni (1986) regarded protection, harmomegathy and ion exchange as the principal functions of the aperture. Additionally apertures act as exits for recognition proteins (Moore *et al.* 1991). However this function could also be performed by the lumina of a reticulate tectum. Horner and Pearson (1978) reported about substances held within the exine and being released from the pollen grain via the lumina in the tectum of the pollen grains of sunflower (*Helianthus annus*).

Sculpturing of the pollen grains is perceived to be associated with the adhesion to pollination vectors. Cases where such relationships seem to exist have been observed, however, they are often complicated (Moore *et al.* 1991). Psilate grains are associated

with anemophily, whereas highly sculptured grains are associated with entomophily. This is often true, but not always (Chaloner 1976). For example, Grayum (1986) studied pollen from the monocotyledonous family Araceae and found that species with verrucate, striate, and reticulate pollen grains were pollinated by a wide range of insects including flies, beetles and bees. Species with psilate or echinate grains, in contrast, were pollinated mainly by beetles (psilate grains) or by flies (echinate grains). Grayum (1986) found spiny grains to be better adapted for the hairy bodies of fast moving insects like flies, whereas the psilate grains become stuck to the smooth surfaces of slow moving beetles with the aid of sticky secretions from the stigmas. Additionally many entomophilous pollen grains are equipped with their own adhesive materials termed pollenkit (Moore *et al.* 1991). Corbet *et al.* (1982) found that electrostatic forces are involved in the transfer of pollen grains. When charged bodies (bees) approach a flower of oilseed rape (*Brassica napus*), they induce an opposite charge in the floral parts. The highest charge is found on the stigma and the lowest on the anther, therefore pollen on the bee's surface will be attracted to the stigma. Smooth grain would rapidly lose their charges when landing on a new surface, whereas a sculptured grain would retain it for longer, and is thus more adhesive. Ornamented grains will therefore stick to an insect vector as well as become adhesive to its final destiny, the stigma (Chaloner 1986).

An understanding of pollination mechanisms in angiosperms aids the understanding of the evolution of angiosperms (Crepet 1979). It is estimated that more than 90 % of angiosperm species are pollinated by animals, especially insects (Wilcock & Neiland 2002), while wind pollination is found in 18 % of the angiosperm families (Culley *et al.* 2002). Wind pollination is regarded as the derived condition, since phylogenetic analysis suggests insect pollinated ancestors for many wind pollinated species, and pollination in many basal angiosperms is performed by beetles and flies (Culley *et al.* 2002). Wind pollination is more common at higher latitudes and elevations (Wilcock & Neiland 2002), probably because insects are limited at such locations. Bees, dipteran flies and small beetles were observed pollinating two American *Oxalis* taxa, *O. alpina* (Rose) Knuth and *O. suksdorfii* Trel. (Weller 1981, Ornduff 1964). There is limited information on the pollination syndrome of the South African *Oxalis* species, but there are indications of insect pollination (Dreyer 1996). South African *Oxalis* species flower during winter, when there are limited numbers of insects around and active. Dreyer

(1996) discussed a number of observations indicating towards the existence of specialized pollination syndromes in South African *Oxalis*.

Oxalis exhibits tristyly and it is relevant to ask what effect tristyly has on pollen morphology and pollen fertility. Tristyly is an adaptation that ensures cross-pollination and reduces lost mating opportunities associated with self-interference, especially geitonogamy (Barrett *et al.* 2000). Tristyly involves varying lengths of stamens and styles, physiological self- and intramorph incompatibility and a number of associated polymorphisms of pollen and stigma characters (Eckert and Barett 1994 a, b & c). When the syndrome is fully expressed, pollen grains from long stamens are the largest and grains from short stamens are the smallest in size (Trognitz *et al.* 2000). Pollen grains from long stamens are only compatible with long styles and the same principle applies to mid and short level stamens and styles. Overall high pollen fertility was recorded from the tristylous Oca (*Oxalis tuberosa* Mol.), but there was a varying degree of fertility associated with the stylar morphs. Pollen from short stamens had the highest fertility and pollen from long stamens had the lowest fertility (Trognitz *et al.* 2000). So one could conclude that, generally, pollen grain size and shape may affect the mode of transportation during pollination. Apertures and exine structures, like the lumina, play a role in the mechanical and physiological functions of the pollen grain. Exine sculptures (e.g. presence or absence of supratectal structures) relate to the type of pollinator the plants use. Therefore there is a fine-tuning between pollen grain morphology, function and reproductive characters (including pollination and pollinator relationships) during the evolution of plants.

Present study

The present study aims to extend the palynological study of *Oxalis* by including some representative American members of the genus. This is important, since a better understanding of both centres of diversity will eventually lead towards the phylogenetic reconstruction of the entire genus. Knowledge of the palynological diversity of both of these centres of diversity can constitute an important first step towards this goal.

Objectives

The main objectives of the present study are:

- To study the pollen type variation in selected American species of *Oxalis*.
- To compare the pollen types observed from the two centres of diversity (American and South African).
- To assess which centre of diversity contains the palynologically more derived species of *Oxalis*.

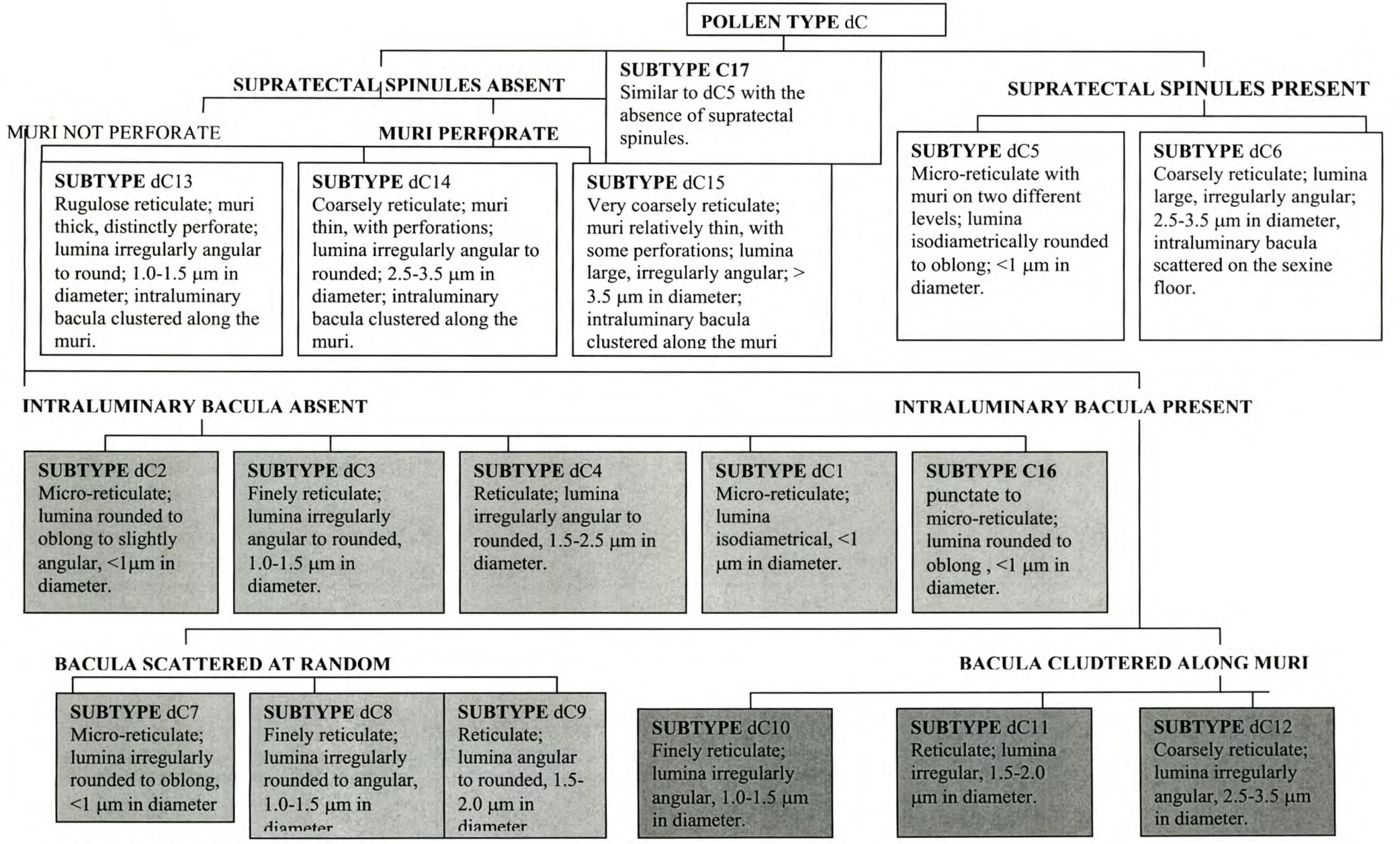


Figure 1.2. Cluster diagram of the subtypes of pollen type dC.

2 MATERIALS AND METHODS

For the present study 50 Pollen samples of American *Oxalis* species were collected from Kew herbarium (K), Royal botanic gardens, Kew, London (Dreyer, *pers. com.*). Specimens were selected to be as representative as possible of all sections of American *Oxalis* taxa (Table 2.1).

The dry anthers were suspended in 96% ethanol to break the anther walls and thus release the pollen grains. Droplets of ethanol containing pollen grains were pipetted onto brass stubs and allowed to dry in a closed petri-dish to prevent dust and dirt from landing on the stubs. The stubs were then sputter coated with a gold-palladium layer and studied with the aid of a Leo 1430 vp scanning electron microscope. Scanning electron micrographs were taken at a fixed magnification of 10 500X at a working distance of 7 mm or at 30 000X at a working distance of 14 mm. These images were used for comparative studies.

Acetolysis was necessary for the species marked by an asterisk in (Table 2.1). The acetolysis procedure proposed by Radford *et al.* (1974) was followed with some modification. The pollen grains were suspended in 96% ethanol, placed in test tubes and left uncovered for 24 hours to allow the evaporation of the ethanol. Pollen grains were then suspended in glacial acetic acid for one hour at room temperature, centrifuged and decanted. To each tube 5ml of 9:1 solution of acetic acid anhydrite and sulphuric acid was added, after which the tubes were heated in a 100^0 C water bath for 10 minutes, stirring each tube occasionally with allocated glass rods. Samples were allowed to cool in cold water, rinsed three times with distilled water and finally rinsed again with 95% ethanol. Pollen grains were then mounted onto brass stubs to be studied under the scanning electron microscope.

In order to assess the tectum structure for *O. magellanica* the pollen wall was studied with the aid of TEM. Since unacetolysed material with intact anthers was necessary for the preparation, a different sample was used (indicated in Table 2.1 by **). Anthers were fixed in 2.5% glutaraldehyde solution in a 0.075 M phosphate buffer at a pH of 7.4 for two days. They were then fixed in a watery 0.1% OsO_4 solution for one hour, dehydrated in a graded acetone series and embedded in epoxy resin. Ultra-thin sections

of pollen grains were cut using a glass knife on a Reichert ultracut microtome. The sections were stained with uranyl acetate and lead citrate. These sections were studied with a ZeissEM 109 transmission electron microscope.

Measurements of the diameters of the lumina and the thickness of the muri were taken from the mesocolopial regions of the micrographs of the pollen grains using a ruler. Pollen grains from the different stylar whorls were not collected separately. Therefore, pollen grain size was not compared in this study. Similarly, pollen grain shape was not included in this study, since it was found to be of minor importance in *Oxalis* by Dreyer (1996). Additionally pollen grain shape varies considerably within one pollen type or species, and is affected by extraction methods and embedding media (Moore *et al.* 1991).

Table 2.1. List of specimens studied in the present study. Sample numbers, specis names and taxonomic positions, collectors and collection numbers and locality are given.

	Sample number	Species	Section	Collector	Collection number	Locality
1	K 16.1	*O. arenaria* Bertero	*Ionoxalis*	Stafford	261	Peru
2	K 193.1	*O. penicillata* Phil.	*Capillares*	Reed	*s. n.*	Chile
3	K 8.1	(*O. californica* (Abrahams) Knuth Volgens Lourteig)	*Corniculatae*	Baker	4175	South California
4	K 15.1	*O. arbuscula* Burm.	*Fruticulosae*	Werdermann	400	Chile
5	K 14.1*	*O. ciliata* Progel	*Holophyllum*	Brade	20654	Brazil
6	K 67.1	*O. divergens* Benth.	*Polyoxalis*	Smith	2109	Guatemala
7	K 99.1	*O. goyazensis* Turcz.	*Thamnoxys*	Harley *et al.*	4576	Brazil
8	K 116.1	*O. hispidula* Zucc.	*Ionoxalis*	Pedersen	6010	Paraguay
9	K 139.1	*O. latifolia* H.B.K.	*Ionoxalis*	Balls	5377	Mexico
10	K 125.2	*O. insignis* Sprague	*Thamnoxys*	Sprague	232	Colombia
11	K 165.1	*O. micrantha* Bert. ex Colla	*Laxae*	Bridges	335	Chile

12	K 171.1	*O. myriophylla* St. Hil.	*Myriophyllum*	Lindeman en de Haas	2668	Brazil
13	K 191.2	*O. peduncularis* H.B.K.	*Carnosae*	Solomon	6691	Bolivia
14	K 190.1	*O. paulosa* St. Hil.	*Andicolae*	Hassler	2625	Paraguay
15	K 208.1	*O. praetexta* Progel	*Holophyllum*	Duser	15694	Brazil
16	K 242.1*	*O. sepium* St. Hilaire	*Thamnoxys*	Glaziou	9378	Brazil
17	K 227.2	*O. rugeliana* Urb.	*Antillanae*	Liogier	15269	Spain
18	K 45.1	*O. clematodes* Donnell Smith	*Clematodes*	John Donnell Smith	2992	Guatemala
19	K 17.2	*O. articulata* Savig.	*Articulatae*	Gibert	454	Uruguay
20	K 20.2	*O. aureoflava* Steudel	*Austro-americanae*	Lobb	136	Chili
21	K 226.2	*O. rufescens* Turcz.	*Andicolae*	Lehmann	4660	Ecuador
22	K 281.1	*O. vallicola* Rose	*Ionoxalis*	Pringle	8540	Mexico
23	K 54.1	*O. cuernavacana* (Rose) Knuth	*Polyoxalis*	Hinton	7906	Mexico
24	K 68.1	*O. dolichopoda* Diels	*Myriophyllum*	Eyerdam	25398	Bolivia

25	K 267.1	*O. tetraphylla* Cav.	*Polyoxalis*	Hawkes	1634	Mexico
26	K 2.1	*O. acetosella* L.	*Acetosellae*	Samuelsson	1103	Sweden
27	K 87.2	*O. filiormis* H.B.K.	*Austro-americanae*	Bogota	3758	Ecuador
28	K 180.1*	*O. obtriangulata* Max.	*Acetosellae*	Iankovskii	2355	Russia
29	K 155.1	*O. lotoides* H.B.K.	*Clematodes*	Asplund	6170	Ecuador
30	K 278.1	*O. tuberosa Molina Var. unduavensis* (Rusby)	*Ortgieseae*	Buchtien	8978	Bolivia
31	K 46.2	*O. compacta* Gill. ex Hook. et Arnott	*Alpinae*	Comber	91	Argentina
32	K 74.2	*O. enneaphylla* Cav.	*Palmatifoliae*	Vallentin	*s.n.*	Fatherland islands
33	K 220.1	*O. refracta* St. Hil.	*Corniculatae*	Pedersen	6156	Argentina
34	K 251.1	*O. squamosa-radicosa* Steud.	*Palmatifoliae*	Tweedie	33	Argentina
35	K 247.2	*O. spiralis* R. et P. ex Don spp. spiralis	*Ortgieseae*	Badcock	466	Bolivia
36	K 81.1	*O. erythrorhiza* Gill. et Hook. et Anott	*Capillares*	Comber	275	Argentina

37	K 95.2	*O. gigantean* Barn.	*Carnosae*	Worth & Morrison	16143	Chile
38	K 123.1	*O. incana* Ph.	*Alpinae*	Philippi	1861	Chili
39	K 150.2	*O. liniflora* Progel	*Articulatae*	Schwarz	1139	Argentina
40	K 140.1	*O. laxa* Hook. et Arn. *Var. hispidissima* Barnéoud	*Laxae*	Biese	1896	Chile
41	K 225.2	*O. rosea* Jacq.	*Roseae*	Morrison	16825	Chile
42	K 256.2	*O. strictula* Steudel	*Carnosae*	Philippi	s.n.	Chili
43	K 168.2	*O. mollissima* (Rusby) Knuth	*Clematodes*	Mandon	851	Bolivia
44	K 73.2	*O. elsae* Knuth	*Myriophyllum*	Mackee	20744	New Caledonia
45	K 176.1	*O. novae-caledoniae* Knuth et Scltr. Ex Knuth	*Myriophyllum*	Mc Kee	32966	New Caledonia
46	K178.1	*O. obliquae* (Rose) Knuth	*Ionoxalis*	Hinton	974	Mexico
47	K 206.2	*O. pintorium* Small	*Thamnoxys*	Ekman	Stockholom 10682	Cuba
48	K 263.2*	*O. tenuicalycula* Knuth	*Thamnoxys*	Hatschbach	25035	Brazil
49	K 22.2	*O. barreleri* L.	*Thamnoxys*	Whistler	2099	Samoa

50	K 160.1*	*O. magellanica* Forst.	*Acetosellae*	Kuschel	743	Chile
51	K 160.2**	*O. magellanica* Forst.	*Acetosellae*	Hoogland & Pullen	5681	New guinea

NB: Specimens marked * were acetolysed and those marked ** were studied with the TEM.

3 RESULTS

The present work is an extension of the palynological work by Dreyer (1996); therefore the classification system of pollen types and subtypes proposed by her is used as far as it is applicable. When new types were described they were accommodated in and thus added to Dreyer (1996) system. Such types are printed in bold in the table below.

Table 3.1: A summary of pollen types and subtypes that were observed among the selected American *Oxalis* species included in the present study. Sample numbers, species names and taxonomic positions, lumina diameter and muri thickness and pollen types according to both Dreyer (1996) and Huynh (1969 a & b) are given.

Sample number	Species	Section	Lumina diameter (µm)	Muri thickness (µm)	Dreyer's(1996) Pollen type / subtype	Huynh's(1969 a & b) pollen type
22.2	*O. barrelieri*	*Thamnoxys*	0.59	0.88	**C16**	hD1
99.1	*O. goyazensis*	*Thamnoxys*	0.84	0.44	dC2	hD1
125.2	*O. insignis*	*Thamnoxys*	0.78	0.69	dC2	hD1
206.	*O. pintorium*	*Thamnoxys*	0.31	0.56	**C16**	hD1
242.1	*O. sepium*	*Thamnoxys*	0.74	0.54	dC2	hD1
263.1	*O. tenuicalyculata*	*Thamnoxys*	0.37	0.64	**C16**	hD1
14.1	*O.ciliata*	*Holophyllum*	0.53	0.55	dC2	hD1
208.1	*O. praetexta*	*Holophyllum*	0.84	0.63	dC2	hA1
247.2	*O. spiralis*	*Ortgieseae*	1.94	0.55	dC9	hA1
278.1	*O. tuberosa*	*Ortgieseae*	3.48	0.45	dC6	hA3
15.1	*O. arbuscula*	*Fruticulosae*	0.65	0.64	dC2	hD1
140.1	*O. laxa*	*Laxae*	1.34	0.7	dC10	hA1
165.1	*O. micrantha*	*Laxae*	0.88	0.43	dC2	hA1

225.2	*O. rosea*	*Roseae*	1.34	0.70	dC10	hA1
45.1	*O. clematodes*	*Clematodes*	0.68	0.50	dC2	hA1
155.2	*O. lotoides*	*Clematodes*	2.79	0.61	dC6	hA3
168.2	*O. mollissima*	*Clematodes*	1.62	0.58	dC11	hA3
8.1	*O. californica*	*Corniculatae*	0.54	0.41	dC2	hA1
220.1	*O. refracta*	*Corniculatae*	1.80	0.60	dC9	hA1
227.2	*O. rugeliana*	*Antillanae*	0.69	0.45	dC2	hA1
190.1	*O. paludosa*	*Andicolae*	0.86	0.45	dC2	hA1
226.2	*O.rufescens*	*Andicolae*	1.36	0.56	dC3	hA1
20.2	*O. aureoflava*	*Austro-americanae*	1.34	0.36	dC3	hA1
87.2	*O. filiformis*	*Austro-americanae*	0.95	0.38	**C17**	hA1
68.1	*O. dolichopoda*	*Myriophyllum*	1.97	0.52	dC4	hA4
171.1	*O. myriophylla*	*Myriophyllum*	0.55	0.44	dC2	hA1
73.2	*O. elsae*	*Neocaledonicae*	0.19	0.54	**C16**	hA2
176.2	*O. novae-caledoniae*	*Neocaledonocae*	0.13	0.49	**C16**	hA2
95.2	*O. giganteam*	*Carnosae*	1.09	0.46	dC10	hA1
191.2	*O. peduncutularis*	*Carnosae*	0.99	0.45	dC2	hA1
256.2	*O. strictula*	*Carnosae*	1.89	0.73	dC11	hA1
17.2	*O. articulata*	*Articulatae*	1.01	0.47	dC3	hA1
150.2	*O. liniflora*	*Articulatae*	1.38	0.49	dC10	hA1
81.1	*O. erythrorhiza*	*Capillares*	1.12	0.45	dC10	hA1

193.1	*O.penicilliata*	*Capillares*	0.63	0.61	dC1	hA1
46.2	*O. compacta*	*Alpinae*	0.87	0.57	dC7	hA1
123.1	*O. incana*	*Alpinae*	1.24	0.76	dC10	hA1
2.1	*O. acetosellae*	*Acetosellae*	0.75	0.46	**C17**	hA2
160.1	*O. magellanica*	*Acetosellae*	-	-	**E**	hC6
180.1	*O. obtriangulata*	*Acetosellae*	0.99	0.38	**C17**	hA2
74.2	*O. enneaphlla*	*Palmatifoliae*	1.78	0.46	dC9	hA1
251.1	*O. squamosoa-radicosa*	*Palmatifoliae*	1.95	0.50	dC9	hA3
16.1	*O. arenaria*	*Ionoxalis*	0.50	0.66	dC1	hA1
116.1	*O. hispidula*	*Ionoxlais*	0.63	0.43	dC2	hA1
139.1	*O. latifolia*	*Ionoxalis*	0.78	0.69	dC2	hA1
178.1	*O. obliquae*	*Ionoxalis*	0.40	0.64	**C16**	hA1
281.1	*O. vallicola*	*Ionoxalis*	1.38	0.36	dC3	hA1
67.1	*O. divergens*	*Polyoxalis*	0.88	0.46	dC2	hA1
54.1	*O. cuernavacana*	*Polyoxalis*	1.50	0.51	dC4	hA1
267.1	*O. tetraphylla*	*Polyoxalis*	1.83	0.46	dC4	hA1

3.1 Description of Pollen types

Pollen type dC: Semitectate reticulate pollen type

Subtype dC1 (Figure 3.1):

Included species (2): *O. arenaria*, *O. penicillata.*

Pollen semitectate, tectum micro-reticulate, hetrobrochate. **Lumina** isodiametrically rounded. Predominant lumina diameter 0.50 µm–0.63 µm, lumina size decreasing greatly towards colpi, forming a distinct colpus margin. **Intraluminary bacula** absent from mesocolpial region. **Muri** smooth, predominant muri thickness 0.61 µm-0.66 µm. **Apertures** tricolpate, colpus membrane sparsely to coarsely granular.

Subtype dC2 (Figure 3.2):

Included species (16): *O. californica, O. arbuscula, O. ciliata, O. divergens, O. goyazensis, O. hispidula, O. latifolia, O.insignis, O. micrantha, O. myriophylla, O. pedunctularis, O. paludosa, O. praetexta,* O. *sepium, O. rugeliana, O. clematodes.*

Pollen semitectate, tectum micro-reticulate, heterobrochate. **Lumina** ranging from rounded, oblong, slightly angular, linear, irregular to irregularly angular. Predominant lumina diameter ranges from 0.53-0.99 µm, lumina size decreasing towards colpi in some species to form a distinct colpus margin. **Intraluminary bacula** absent from mesocolpial region. **Muri** smooth, predominant muri thickness 0.41–0.68 µm. **Apertures** tricolpate in the majority of species, with only a few species displaying tetracolpate grains. Colpus membrane coarsely to sparsely granular.

Sub-type dC3 (Figure 3.3):

Included species (4): *O. articulata, O. aureoflava, O. rufescens, O. vallicola.*

Pollen semitectate, tectum finely reticulate to reticulate (O. *articulata*), hetrobrochate. **Lumina** ranging from rounded to oblong to irregular to irregularly angular. Predominant lumina diameter ranges from 1.01–1.38 µm, lumina size decreasing towards the colpi, except in *O. rufescens.* **Intraluminary bacula** absent from the mesocolpial region. **Muri** smooth, predominant muri thickness ranges from 0.36–0.56 µm. **Apertures** tricolpate in all species, except in *O. vallicola*, where there can also be tetracolpate, hexacolpate and pantocolpate grains in addition to tricolpate ones. Colpus membrane coarsely to moderately granular.

Subtype dC4 (Figure 3.4):

Included species (3): *O. cuernavacana, O. dolichopoda, O. tetraphylla*

Pollen semitectate, tectum reticulate with the exception of *O. tetraphylla,* which displays a finely reticulate tectum, hetrobrochate with the exception of *O. dolichopoda*, which is homobrochate. **Lumina** irregularly angular to rounded. Predominant lumina diameter 0.83–1.97 µm. **Intraluminary bacula** absent from the mesocolpial region. Muri smooth, predominant muri thickness 0.46–0.52 µm. **Apertures** tricolpate. **Colpus membrane** coarsely to moderately granular.

Subtype dC6 (Figure 3.5):

Included species (2): ***O. lotoides***, ***O. tuberosa.***

Pollen semitectate, tectum coarsely reticulate, homobrochate. **Lumina** irregularly angular. Predominant lumina diameter ranges from 2.97–3.48 µm. **Intraluminary bacula** free-standing on nexine floor. **Muri** smooth to stratified with supratectal spinules, predominant muri thickness 0.45–0.61 µm. **Apertures** tricolpate. Colpus membrane moderately granular.

Subtype dC7 (Figure 3.6):

Included species (1): *O. compacta.*

Pollen semitectate, tectum micro-reticulate, heterobrochate. **Lumina** irregularly rounded to oblong to slightly angular. Predominant lumina diameter 0.87 µm, lumina size decreasing slightly towards the colpi. **Intraluminary bacula** randomly scattered on the nexine floor. **Muri** smooth, predominant muri thickness 0.57 µm. **Apertures** tricolpate, colpus membrane granular.

Subtype dC9 (Figure 3.7):

Included species (4): *O. enneaphylla, O. refracta, O. squamosa-radicosa*, *O. spiralis*.

Pollen semitectate, tectum reticulate to coarsely reticulate, heterobrochate, except in *O. squamosa-radicosa*, where the tectum is homobrochate. **Lumina** rounded, irregular to angular. Predominant lumina diameter ranges from 1.78–1.94 µm. **Intraluminary bacula** free-standing on nexine floor. **Muri** smooth to stratified, predominant muri thickness 0.46–0.60 µm. **Apertures** tricolpate, with aberrant grains

in *O. ennaeaphulla*. Colpus membrane coarsely to moderately granular, except in *O. squamosa-radicosa*, where the colpus membrane is rugulate.

Subtype dC10 (Figure 3.8):

Included species (6); *O. erythrorhiza, O. gigantean, O. incana, O. liniflora, O. laxa , O. rosea.*

Pollen semitectate, tectum finely reticulate, heterobrochate. **Lumina** rounded to irregularly angular. Predominant lumina diameter ranges from 1.12–1.35 µm, with the lumina diameter decreasing towards the colpi in some species to form a distinct colpus margin. **Intraluminary bacula** associated with the muri. **Muri** smooth to slightly stratified, predominant muri thickness 0.44–0.76 µm. **Apertures** tricolpate, with tetracolpate grains also recorded from *O. liniflora*. Colpus membrane coarsely granular to granular (*O. erythrorhiza*).

Subtype dC11 (Figure 3.9):

Included species (2): *O. strictula, O. mollissima.*

Pollen semitectate, tectum reticulate to finely reticulate, homobrochate. **Lumina** irregular to angular, predominant lumina diameter 1.62–1.89 µm, lumina size decreasing greatly towards the colpi to form a distinct colpus margin. **Intraluminary bacula** associated with the muri. **Muri** smooth, predominant thickness 0.58– 0.73 µm. **Apertures** tricolpate. Colpus membrane moderately to coarsely granular.

Subtype C16 (Figure 3.10):

Included species (6): *O. elsae, O. novae-caledoniae, O. obliquae, O. pintorium, O. tenuicalyculata, O. barreleri.*

Pollen semitectate, tectum punctate to micro-reticulate, homobrochate to heterobrochate. **Lumina** range from isodiametrically rounded to oblong to slightly irregular to linear. Predominant lumina diameter 0.19–0.59 µm, lumina size decreasing towards the colpi. **Intraluminary bacula** absent from mesocolpial region or not visible because of small lumen size and thicker muri. **Muri** smooth,

predominant muri thickness 0.44–0.88 µm. **Apertures** tricolpate, with *O. myriophylla and O. pintorium* also including tetracolpate, hexacolpate and aberrant grains. Colpus membrane non-granular to granular to rugulate.

Subtype C17 (Figure 3.11):

Included species (2): *O. acetosellae*, *O. erythrorhiza, O. filiformis.*

Pollen semitectate, tectum micro-reticulate. heterobrochate. **Lumina** rounded to slightly irregular. Predominant lumina diameter 0.95–0.99 µm, lumina size decreasing towards the colpi. **Intraluminary bacula** absent from mesocolpial region. **Muri** on two different levels. Predominant muri thickness 0.38 µm. **Aperture**s tricolpate, colpus membrane moderately granular.**Pollen type E** (Figure 3.12):

Type E (figure 3.12)

Included species (1): *O. magelanica.*

SEM

Pollen tectate, tectum densely covered with supratectal verrucae. Predomnant verruca width 1.14 µm. Tricolpate, colpus membrane granular.

TEM

Exine ca. 2 µm–3.5 µm thick, comprising of a thick ectectine (supratectal verrucae, tectum, collumellae, foot layer) and a much thinner endexine. **Supratectal verrucae** *ca.* 1 µm long and appear to be independent of collumellae. **Tectum** 0.8 µm thick, elements of tecum rounded to oval in cross section. **Collumellae** 1 µm thick, simplicollumellate; collumellae erect, cylindrical, 0.4 µm thick in cross-section, thickening towards their bases. Not all collumelae are capped by supratectal verrucae, intracollumelar spaces not uniform, some collumellae are close to each other, while others are spaced further apart. **Footlayer** 0.75 µm thick, uneven. **Endexine** thin, continuous, *ca.* 0.4 µm thick.

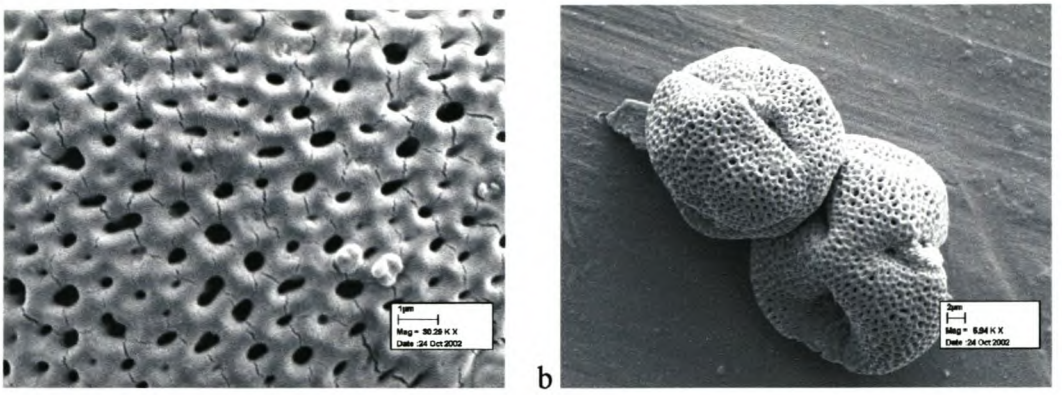


Figure 3.1 a - b. *Oxalis* pollen subtype dC1. a & b: *O. penicillata* (193.1). (a) Mesocolpial region of exine showing micro-reticulate tectum with isodiametrically rounded lumina. (b) Equatorial and polar view of pollen grains.

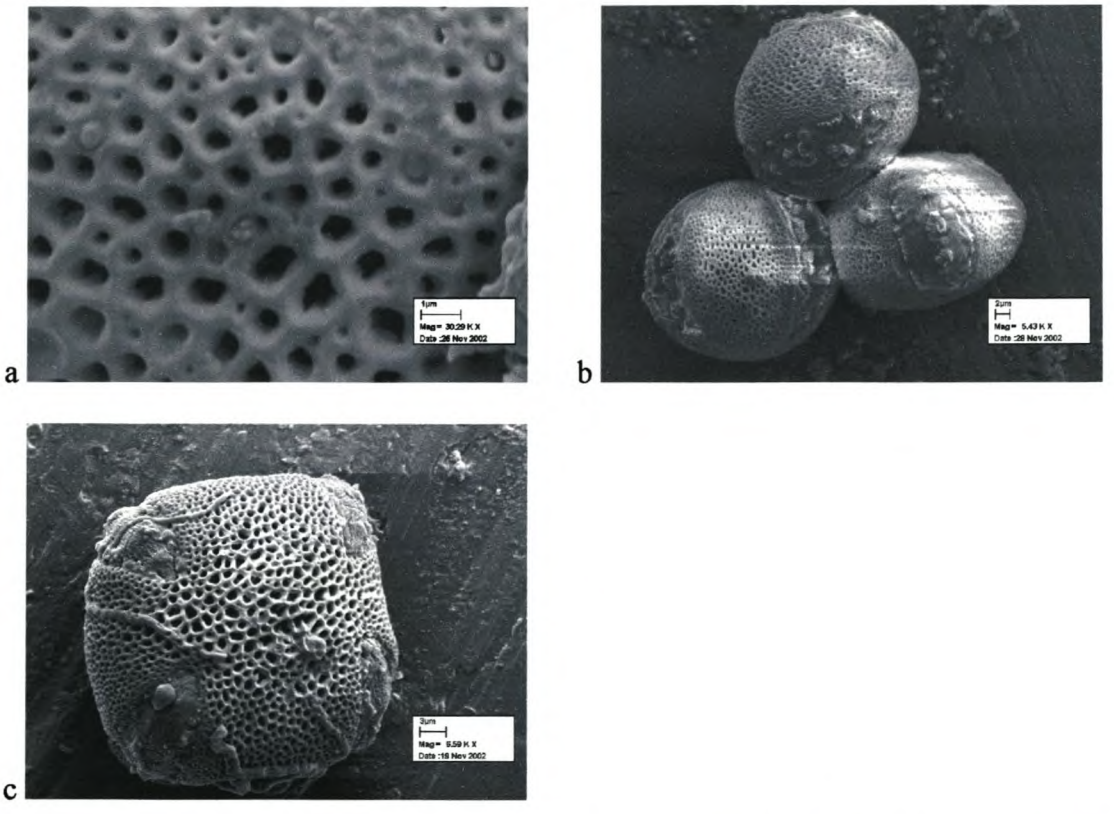


Figure 3.2 a – c. *Oxalis* pollen subtype dC2. (a) *O. clematodes* (45.1), mesocolpial region of exine showing micro-reticlate tectum with rounded to oblong lumina. (b) *O. hispidula* (116.1), equatorial view of pollen grains. (c) *O. paludosa* (190.1), polar view of tetracolpate grain.

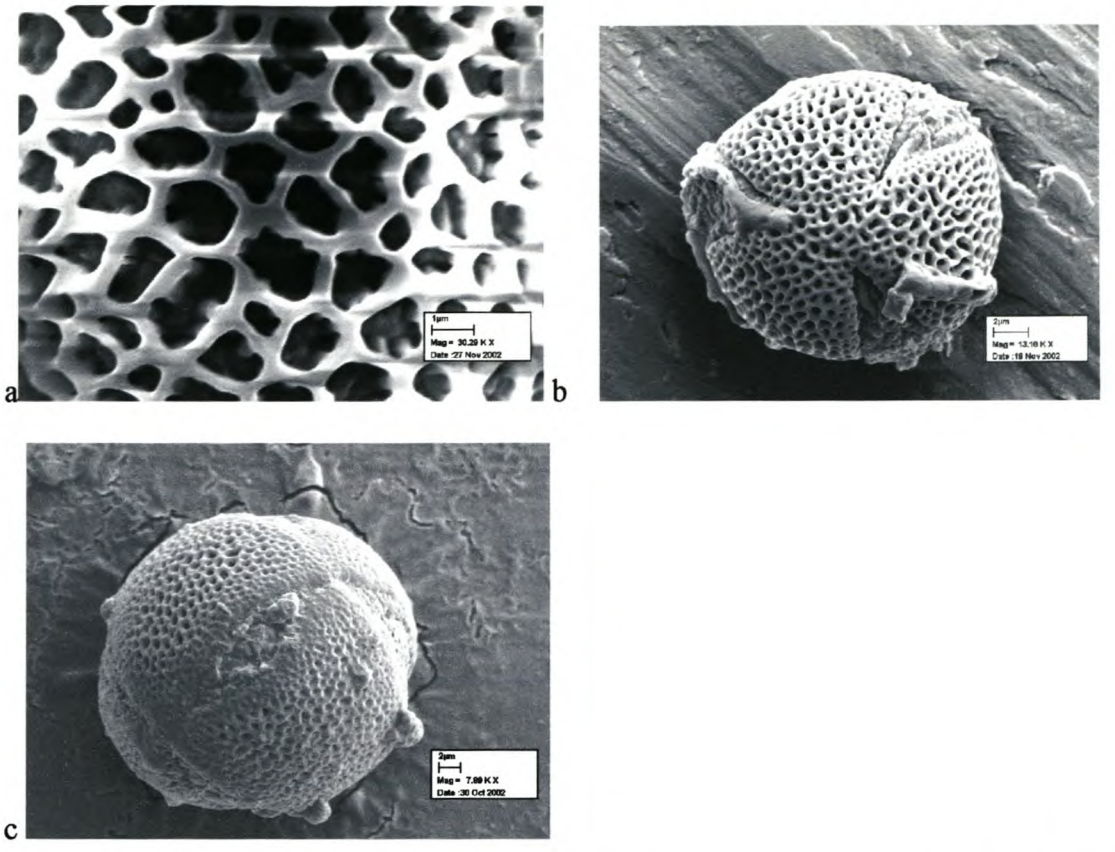


Figure 3.3 a – c. *Oxalis* pollen subtype dC3. (a) *O. aureoflava* (20.2), mesocolpial region of exine showing finely reticulate tectum with round to irregularly angular lumina. (b) *O. articulata* (17.2), polar view of pollen grain. (c) *O. vallicola* (281.1), hexacolpate pollen grain.

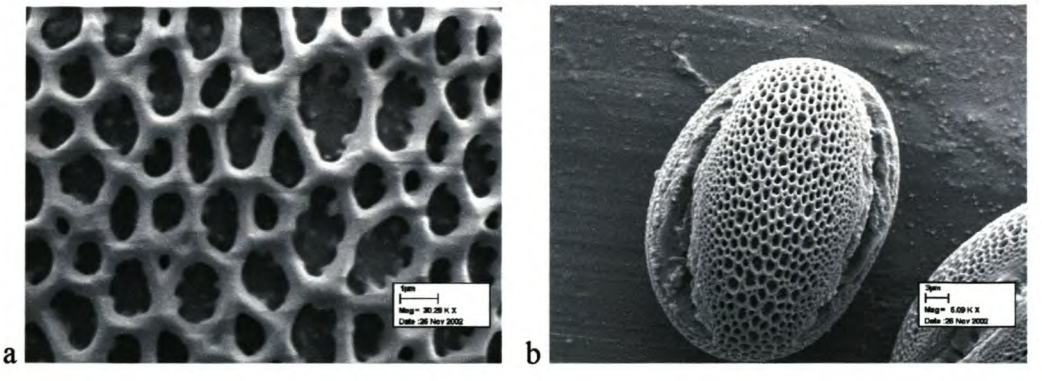


Figure 3.4 a – b. *Oxalis* pollen subtype dC4. a & b *O. tetraphylla* (267.1). (a) Mesocolpial region of exine showing finely reticulate tectum with rounded to irregularly angular lumina. (b) Equatorial view of pollen grain.

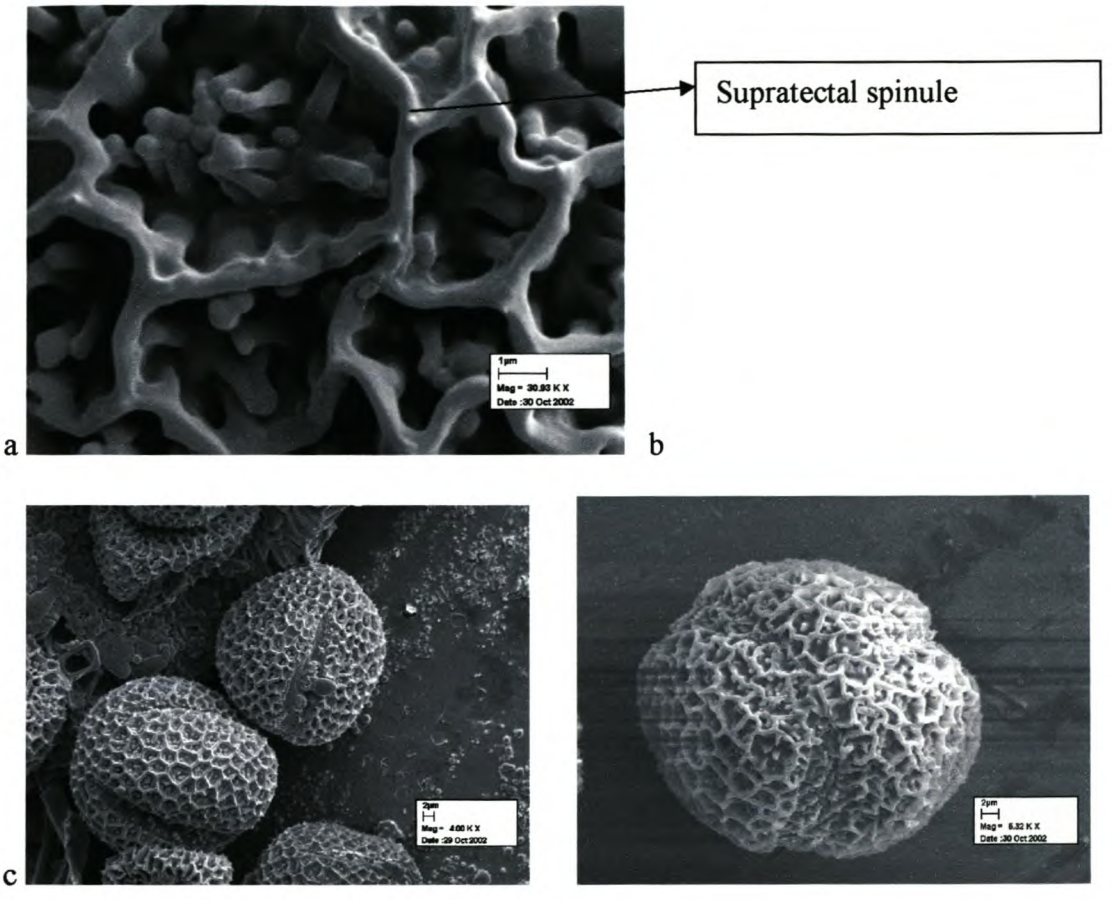


Figure 3.5 a – c. *Oxalis* pollen subtype dC6. a & b *O. lotoides* (155.1). (a) Mesocolpial region of exine showing coarse, reticulate tectum with supratectal spinules and free standing intraluminary bacula. (b) Polar view of pollen grain. (c) *O. tuberosa* (278.1) equatorial view of pollen grain.

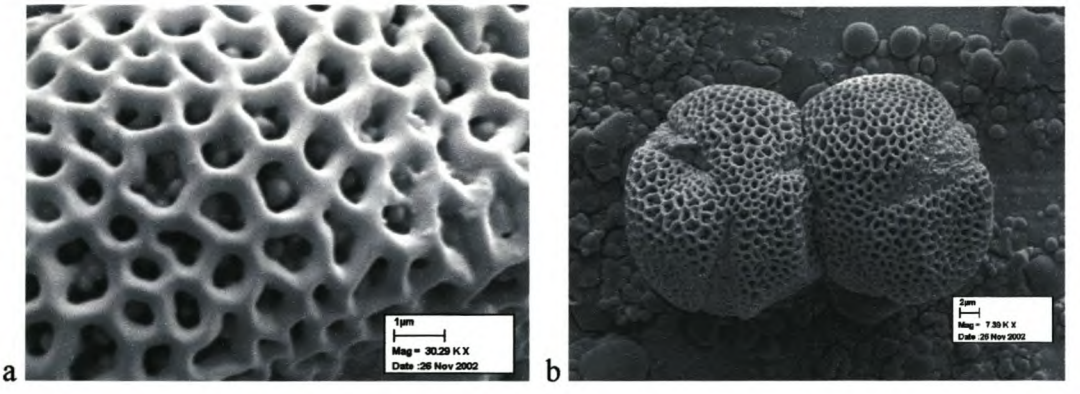


Figure 3.6 a – b. *Oxalis* pollen subtype dC7. a & b *O. compacta* (46.2). (a) Mesocolpial region of exine showing micro-reticulate tectum with rounded to angular lumina and randomly scattered intraluminary bacula on the nexine floor. (b) Polar view of pollen grain.

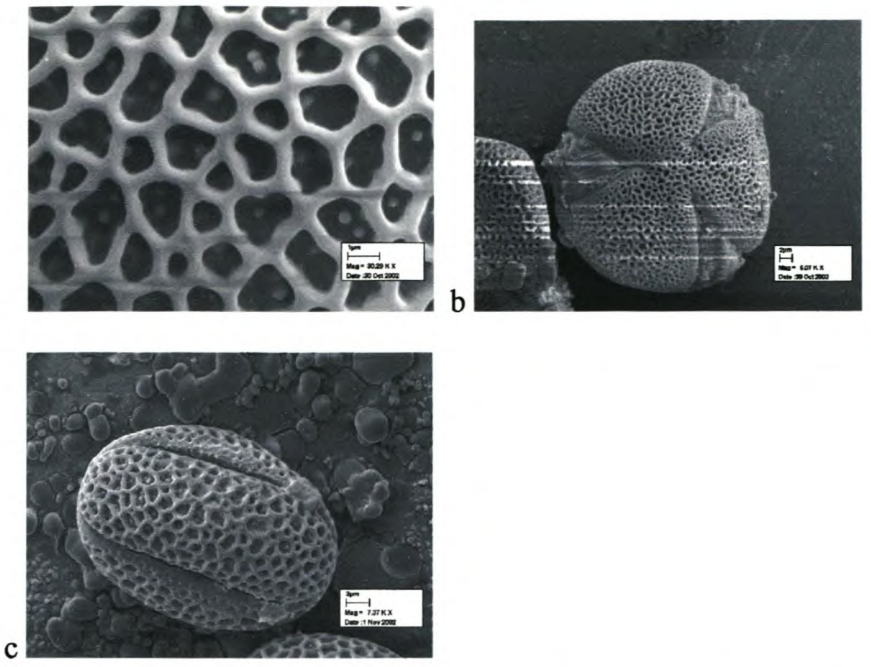


Figure 3.7 a – c. *Oxalis* pollen subtype dC9. a & b, *O. enneaphylla* (74.2). (a) Mesocolpial region of exine showing reticulate tectum with irregular to angular lumina. (b) Pollar view of pollen grain. (c) *O. squamosa-radicosa* (251.1), equatorial view of pollen grain.

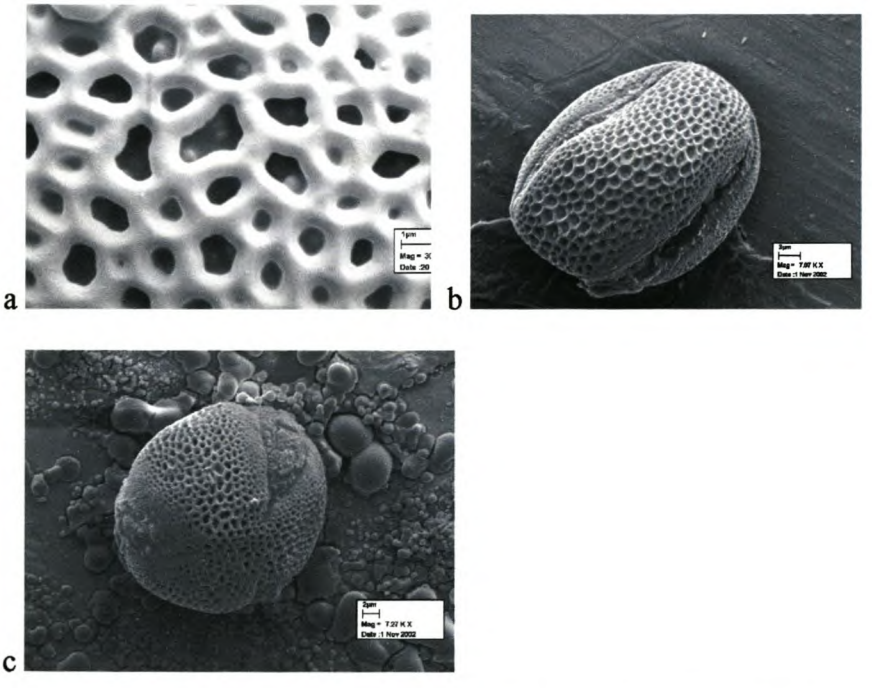


Figure 3.8 a – c. *Oxalis* pollen subtype dC10. (a) *O. incana* (123.1), mesocolpial region of exine showing finely reticulate tectum with rounded to irregularly angular lumina and clustered intraluminary bacula. (b) *O. roseae* (225.2). Equatorial view of pollen grain. (c) *O. roseae* (225.2). Polar view of pollen grain.

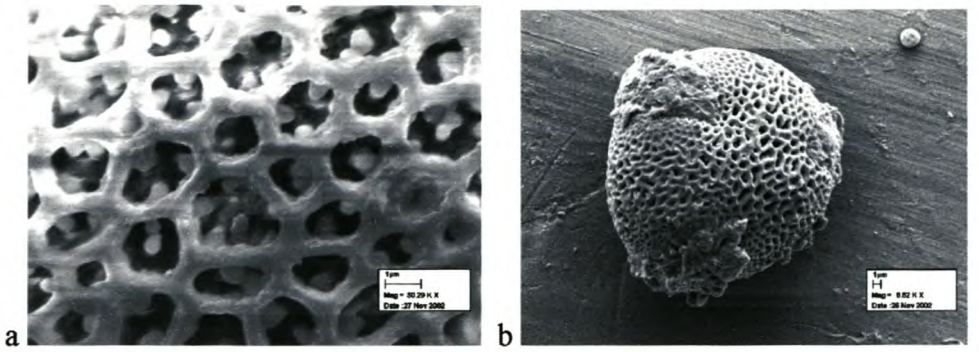


Figure 3.9 a – b. *Oxalis* pollen subtype dC11. (a) *O. mollissima* (168.2), mesocolpial region of exine showing reticulate tectum with irregularly angular lumina and clustered intraluminary bacula. (b) *O. strictula* (256.2), polar view of pollen grain.

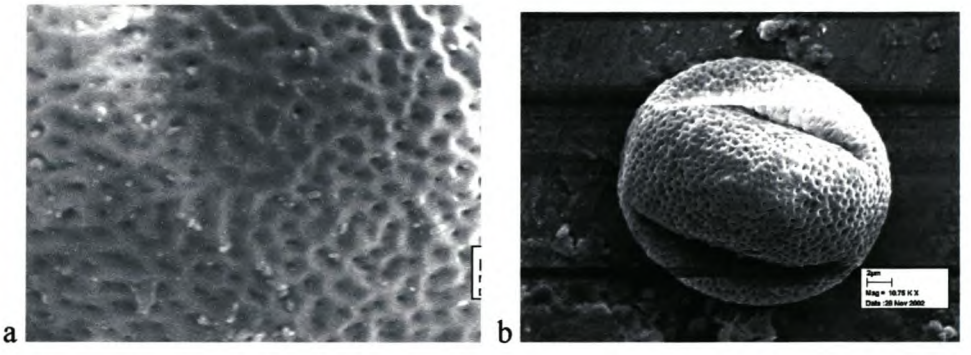


Figure 3.10 a – b. *Oxalis* pollen subtype **C16**. (a) *O. novae-caledoniae* (176.1), mesocolpial region of exine showing punctate tectum. (b) *O. obliquae* (178.1), equatorial view of pollen grain.

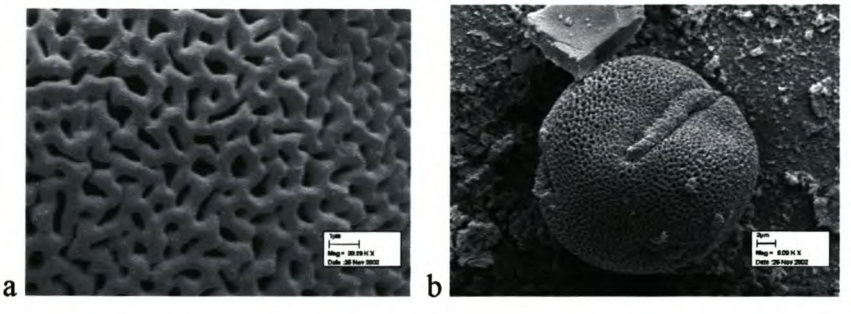


Figure 3.11 a – b. *Oxalis* pollen subtype **C17**. a & b: *O. obtriangulata* (180.1). (a) Mesocolpial region of exine showing micro-reticulate tectum and muri arranged on two different levels. (b) Polar view of pollen grain.

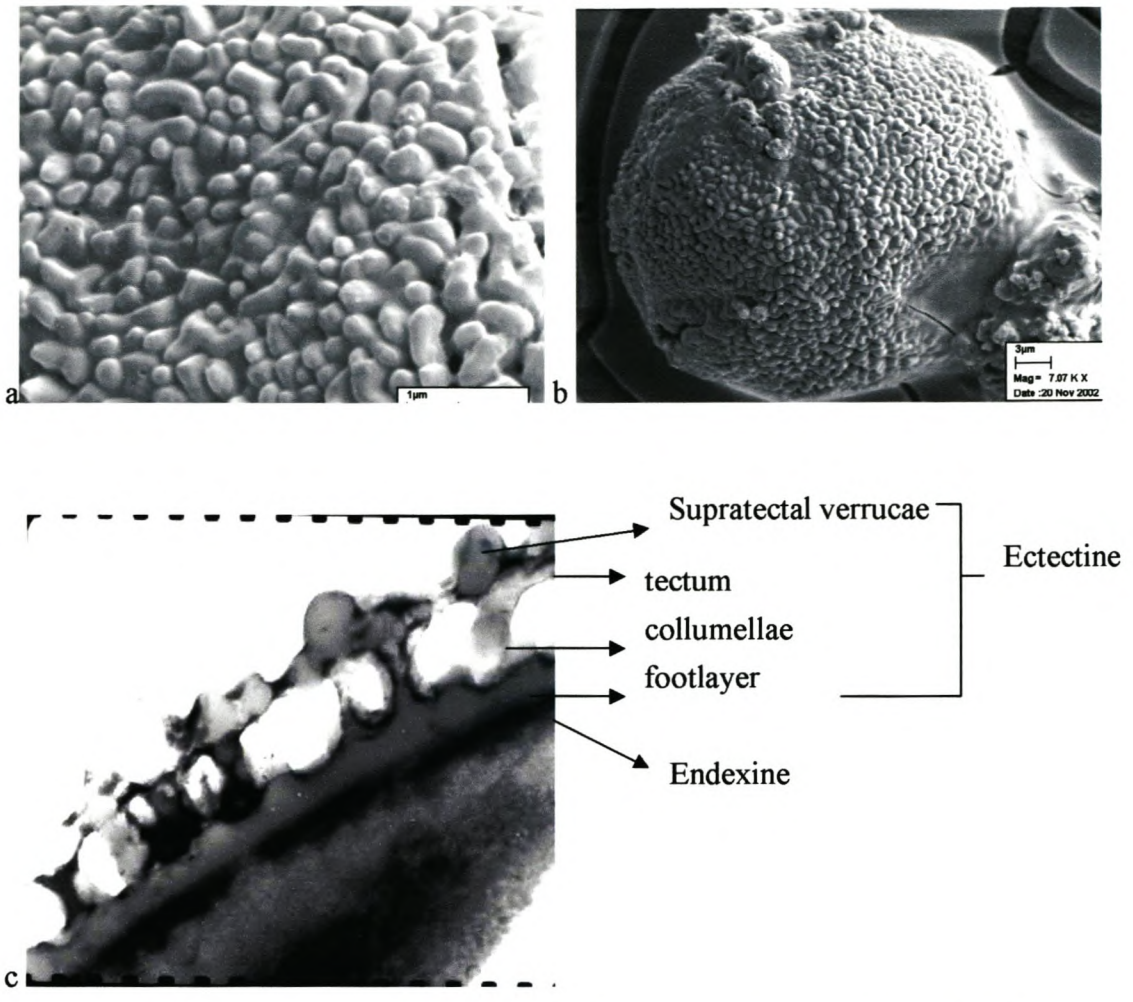


Figure 3.12 a – b. *Oxalis* pollen type **E**. a & b: *O. magellanica* (160.1), SEM. The bar measures 1µm. (a) mesocolpial region of exine showing tectum densely covered by supratectal verrucae. (b) Polar view of pollen grain. (c) *O. magellanica* (160.2), TEM, wall section of pollen grain showing the ectectine and endexine.

3.2 Pollen types recorded from present study

The first objective of the present study was to assess pollen type variations among selected American species of *Oxalis*. Huynh (1969 a & b) identified four (hA, hB, hC and hD) pollen types and 13 pollen subtypes. Dreyer (1996) used SEM and TEM techniques that allowed for proper three dimensional images of the exine structure. This enabled better definitions of the pollen types (Table 3.1). Dreyer (1996) widely defined Huynh's (1969 a & b) pollen type hA1 as the reticulate pollen type dC, but subdivided it into a number of subtypes, namely dC1- dC4 and dC7 - dC15.

Table 3.2 A comparison of the pollen types identified by Huynh (1996 a & b), Dreyer (1996) combined and compared to the results of the present study.

Pollen types classification according to Huynh (1969 a & b)	Pollen types classification according to Dreyer (1996)	Pollen types identified in the present study
hA1	dC1, dC2, dC3, dC4, dC7, dC8, dC9, dC10, dC11, dC13 & dC15	dC1, dC2, dC3, dC4, dC7, **C17**, dC9, dC11 & **C16**
hA2	--	**C16 & C17**
hA3	C8, C14	dC6, dC9 & dC11
hA4	--	dC4
hC1-hC3	A	--
hC4	D	--
hC5	B	--
hC6	--	**E**

NB: -- represents pollen types and subtypes not recorded in the respective studies.

A total of 11 different pollen types and subtypes were recorded from the 50 American *Oxalis* species selected for the present study. Eight of the subtypes namely dC1, dC2, dC3, dC4, dC6, dC7, dC9, dC10 and dC11 were also recorded from the South African members by Dreyer (1996). However, three pollen types recorded from ten of the species represented in this study were not found among the South African members of *Oxalis*. These are subtypes **C16**, and **C17,** recorded from six and three species respectively, and pollen type **E** recorded from a single species. Huynh (1969 a & b) also recorded uniquely American pollen types in nine of these species (Table 3.2).

4 DISCUSSION

4.1 Distribution and possible taxonomic significance of pollen types within the American sections of *Oxalis*

The distribution of pollen types within the sections is summarized in Table 4.1. The number of representative species from each section is, however, very small. The taxonomic discussions derived from these results are thus tentative and must be validated by further studies.

Dreyer (1996) clustered the reticulate subtypes of pollen type dC into the following groups: dC1 – dC4, dC7 – dC9 and dC10 – dC12. She also regarded the first two groups as being closely related. This view is also followed in the present study with the addition of **C16** (Figure 3.10) to the dC1 – dC4 group. The decision to include **C16** in the group dC1 – dC4 is based on the observation that this subtype also has a similar semitectate, reticulate exine structure with smaller lumina and proportionately thicker muri. This subtype is most similar to Dreyer's (1996) pollen subtype dC2 (Figure 3.2). Pollen subtypes dC10 (Figure 3.8) and dC11 (Figure 3.9) are regarded as closely related, while pollen subtype dC12 was not recorded in the present study.

Pollen subtype dC6 (Figure 3.5) is more complex than the rest of the pollen subtypes in the present study, because it displays well-developed supratectal spinules. This subtype was regarded as monotypic among the South African members of *Oxalis*, but in the present study it was recorded from two species belonging to different sections (Table 4.1).

Pollen subtype **C17** (Figure 3.11) differs markedly from the other pollen subtypes in the present study, having muri separated onto two different levels. Pollen subtype **C17** looks more similar to the monotypic dC5 that was recorded in *O. levis* Salter from the South African section *Angustatae* subsection *Linearis*.

Pollen type **E** (Figure 3.12) is completely different from the rest of the subtypes, because it has a complete tectum and possesses supratectal verrucae.

Table 4.1 Distribution of pollen types and subtypes in American sections of *Oxalis*.

NB: The number following the pollen types written in brackets indicates the number of times that particular pollen type occurred within a given section. For example (dC2) 2 means dC2 occurred twice.

	Section	Number of representative species	Pollen types / subtypes
Sections possessing similar (related) pollen subtypes	*Holophyllum*	2	(dC2) 2
	Cornuculatae	2	dC2, dC9
	Andicolae	2	dC2, dC3
	Myriophyllum	2	dC2, dC4
	Neocaledonicae	2	(**C16**) 2
	Palmatifoliae	2	(dC9) 2
	Polyoxalis	3	dC2, (dC4) 2
	Thamnoxys	6	(dC2) 3, (**C16**) 3
	Ionoxalis	5	dC1, (dC2) 2, dC3, **C16**
Sections possessing dissimilar (unrelated) pollen subtypes	*Capilares*	2	dC1, dC10
	Alpinae	2	dC7, dC10
	Laxae	2	dC2, dC10
	Articulatae	2	dC3, dC10
	Carnosae	3	dC2, dC10, dC11
	Ortgieseae	2	dC6, dC9
	Clematodes	3	dC2, dC6, dC11
	Austro-americanae	2	dC3, **C17**
	Acetosellae	3	(**C17**) 2, **E**
	Fruticulosae	1	dC2
	Roseae	1	dC10
	Antillanae	1	dC2

Despite being based on a small number of representative species, sections ***Holophyllum***, ***Cornuculatae***, ***Andicolae***, ***Myriophyllum***, ***Neocaledonicae, Palmatifoliae, Polyoxalis, Thamnoxys*** and ***Ionoxalis*** seem to possess similar pollen subtypes. According to Huynh (1969 a & b) sections *Thamnoxys*, *Holophyllum*, *Heterophyllum*, *Monoxalis*, *Ionoxalis* and *Polyoxalis* are examples of taxonomically well-defined sections based on phytogeographical data. She also mentioned that there is a close affinity between members of *Thamnoxys*, *Holophyllum*, *Heterophyllum* and *Monoxalis* based on both their distributional patterns and their shared palynological character of possessing colporate pollen grains. Additionally *Ionoxalis* and *Polyoxalis* are the only American sections comprising geophytic species of *Oxalis*, and are probably closely related. Sections *Heterophyllum* and *Monoxalis* were not represented in this study.

Even with the small number of specimens per section studied, sections ***Capilares***, ***Alpinae***, ***Laxae***, ***Articulatae, Carnosae, Ortgieseae, Clematodes, Austro-americanae*** and ***Acetosellae*** includes species with unrelated pollen types and subtypes. This is probably an indication of taxonomic problems in the delimitation of these sections.

Sections ***Fruticulosae, Roseae*** and ***Antillanae*** were represented by a single species each. No palynological patterns could thus be deducted for these sections.

4.2 Comparison between pollen types of the selected American and South African *Oxalis* species

A second aim of the present study was to compare the pollen types of the American species to those of South African species of *Oxalis*. This comparison was possible with regard to:

- The number of pollen types and subtypes observed in the two centres. (Which centre includes the most diverse pollen types and subtypes?).
- The frequency of the pollen types and subtypes in the respective centres of diversity.
- The taxonomic implication of the pollen types and subtypes in the two centres of diversity.
- The occurrence of specialised or derived pollen types and subtypes.

According to Dreyer (1996) pollen type dC is the most common type among South African *Oxalis* species, followed by pollen types dD, dA and finally the unique pollen type dB. Pollen subtype dC2 was the most frequently observed subtype among the South African members of the genus.

Pollen type dC was recorded in all but one of the representative species included in the present study. This single species (*O. magellanica*) was found to possess pollen type **E**, a pollen type not present among the South African members of the genus. Similarly pollen subtype dC2 was the most widely distributed subtype (table 4.1), while dC7 was recorded only once. At this stage pollen subtype dC7 cannot be considered as being monotypic, as only a small number of the American members of *Oxalis* were studied.

Pollen subtypes dC1 – dC4 and dC7 – dC9 were found to co-occur in natural South African sections, and therefore were considered to be taxonomically significant when viewed as a group. To some extent a similar pattern was observed in the present study, where **C16** is included in the dC1 – dC4 subtype group. Pollen subtype group dC1 – dC4 and **C16** co-occur in seven of the American sections (Table 3.2). Subtype dC7 was recorded only once, but it co-occurs with the dC10 pollen type in section *Capilares* (Table 3.1). Subtype dC8 was not recorded from the present study and dC9 was observed co-occurring with dC2 in one section only.

Among the South African members of *Oxalis* pollen subtypes dC10 – dC12 seem to be closely related and therefore regarded as taxonomically significant when considered as a group (Dreyer 1996). Pollen subtype dC12 was not recorded in the present study and pollen subtypes dC10 and dC11 were observed co-occurring with pollen subtypes dC1, dC2, dC3, dC6 and dC7. The co-occurrence of these 'unrelated' pollen subtypes might be an indication of problems in the delimitation of the American sections of the genus.

Each of the pollen subtypes dC5, dC6, dC12, dC13, dC14 and dC15 was only found in a single species and are thus referred to as monotypes in South African *Oxalis*. These subtypes thus have no taxonomic significance, but according to Dreyer (1996) they could indicate an advanced evolutionary state of the taxa in which they occur. Only one (dC6) of the African monotypic pollen subtypes was recorded in the

American species, where it occurs in two species from different sections. This could indicate a misplacement of species in the different sections. Pollen subtype **C17** is very similar to the monotypic subtype dC5 in South Africa, the only difference being that **C17** does not possess supratectal spinules. Further investigations may reveal a close relationship between the American and South African taxa possessing these two pollen subtypes.

Pollen type **E** was recorded from a single American species in the present study. It is the only tectate pollen type observed in the present study and is covered by supratectal verrucae, displaying a complex exine structure. This suggests an advanced evolutionary state of the species in which it occurs and is in accordance with the ideas of Van Campo (1967 & 1976), Muller (1970) and Ferguson & Skvarla (1982), that there is an evolutionary tendency towards an increased complexity of pollen wall structure.

To date more specialized pollen types and subtypes have been observed among the South African members of the genus than among the American ones. According to Dreyer (1996) pollen type dD is the most specialized of all the pollen types, followed by dA, dB and dC. Within pollen type dC, the five monotypic subtypes are considered to be more advanced. According to Muller (1970) there is an evolutionary progression of exine structures from collumella-derived structures (mostly with reticulate architecture) to tectal structures (mostly spinose or verrucose supratectal structures). Therefore in the present study pollen type **E** appears to be more advanced than pollen type dC among the American members of the genus. However, Walker (1976) warned that sculpturing represents a more or less reversible character that should be interpreted in terms of the individual correlations observed within a given taxon.

The South African members of the genus also display a larger diversity of pollen types and subtypes than the American members included in this study. Pollen type dC is the most common and subtype dC2 the most abundantly distributed in both South African and American members of the genus. The presence of more specialized types and subtypes in the South African members of the genus seems to support Huynh's (1969 a & b) view that the South African *Oxalis* are more derived than the American taxa. Further palynological studies, including all (or the majority) of American *Oxalis*

species are necessary for a complete comparison and taxonomic conclusions regarding the two centres of diversity.

4.3 Phylogenetic implications

Attempting to evaluate the two centres of diversity with regard to the occurrence of advanced *Oxalis* species is the final objective of the present study. Information based on other taxonomic characters like morphology is important for this discussion. Morphological distinctions between the American and South African members of the genus have been discussed in previous studies (Knuth 1930). The progressive reduction of the stem, leaf, number of floweres per inflorescence, and the transition from endospermous to exendospermous seed is considered to represent phylogenetically advanced states in the Oxalidaceae in general and *Oxalis* in particular (Reiche 1894; Knuth 1914, 1930 and Salter 1944). Following this view, both Knuth (1930) and Salter (1944) named five South African sections of *Oxalis* as the most advanced taxa in the Oxalidaceae. These sections are: *Sagitatae*, *Campanulatae*, *Latifoliolatae*, *Crassulae* and *Angustatae*. Dreyer's (1996) palynological data supports this view to some extent. The most advanced pollen type dD was found in members of section *Latifoliolatae* and both section *Angustatae* subsection *Sessilifoliatae* and section *Angustatae* subsection *Linearis*. Additionally pollen types dA and dB, which are also considered as more advanced, were both recorded from section *Sagitattae*.

Huynh (1969 a &b) proposed a possible trend in the reduction of the stem which turns from an arborescent to a suffrutescent or herbaceous-caudescent habit in the primitive genera (*Averrhoa*, *Sarcotheca*, *Eichleria* and many *Epiphytum* species) and finally into bulbs in *Oxalis* species. The American section *Thamnoxys* is considered as basal to sections *Holophyllum*, *Monoxalis* and *Heterophyllum,* and all four sections possess suffrutescent stems. Only two American sections, *Polyoxalis* and *Ionoxalis*, possess bulbs and the South American section *Palmatifoliae* is regarded as transition between having and not having bulbs (Huynh 1969 a & b). All indigenous South African *Oxalis* taxa possess true bulbs, but the South African bulbs are believed to have been derived independently to the American bulbs. These structures differ significantly between African and American sections (Salter 1944; Denton 1973). From these observations, Huynh (1969 a & b) concluded that *Oxalis* is the most derived genus in

the Oxalidaceae, and that the American section *Thamnoxys* is the most likely basal section in *Oxalis*. The presence of colporate pollen type hD in the American sections *Thamnoxys*, *Holophylum*, *Heterophylum* and *Monoxalis* is also viewed as reflecting the ancestral condition (Huynh 1969 a & b). However, the few species of sections *Thamnoxys* and *Holopyllum* included in the present study have colpate pollen types. This discrepancy in results of the present study and those of Huynh (1969 a & b) can probably be explained by the different techniques employed in the two studies. Huynh (1969 a & b) used only LM analysis, while SEM analyses were used in the present study. The SEM provides much more detail than the LM, which suggests that Huynh (1969 a & b) might have been mislead during her observations. Further studies, including more species, are necessary to reach a final conclusion on this question.

The tectate pollen type **E**, with its verrucate supratectal structures, is the most specialized pollen type observed from a single species in this study. The remaining 49 species posses the semitectate reticulate pollen type dC, which is common and widely distributed among both American and South African members of the genus. In the South African species pollen type dC is subdivided into 15 subtypes, six of which are monotypic and some that are more advanced than others (Dreyer 1996). Subtypes dC5 and dC6 (Figure 3.5) have a tectum covered with supratectal spinules. These monotypic subtypes are considered to be complex and derived (Dreyer 1996). In the present study pollen subtype dC6 was recorded from two species. This subtype is equipped with supratectal spinules in both South African and American taxa. In South Africa dC6 was recorded from two species belonging to the same section and subsection (Dreyer 1996). The close affinity between these two species was noted, and they were eventually regarded as being conspecific (Dreyer & Van Wyk 1998). In the present study dC6 was observed in two species belonging to different sections. In section *Ortigeseae* it co-occurs with pollen subtype dC9 and in section *Clematodes* it co-occurs with dC2 and dC11. Huynh (1969 a & b) comments on the possible taxonomic problems of Knuth's (1930) classification system and particularly speculates that some sections including, *Clematodes*, may be taxonomically poorly defined. The co-occurrence of three unrelated pollen subtypes (dC2, dC6 and dC11) may also be a confirmation of the taxonomic problems in the delimitation of section *Clematodes*.

Subtype **C17** occurs in three species, two of which belong to one section. This pollen subtype is very similar to dC5 of the South African species, except that it does not possess supratectal spinules. In the South African species pollen subtype dC5 is characterized by having muri at different levels and a tectum covered with small blunt supratectal spinules. Subtype **C17** also has muri on two different levels, but no clear supratectal spinules are present. Dreyer (1996) regarded pollen types with supratectal structures as more complex than those without them and that a gradual increase in tectum complexity represents a progressive evolutionary trend. Therefore considering the similarity between **C17** in the American species and dC5 in the South African species and the monotypic state of dC5 in South Africa one may speculate that dC5 was derived from the American pollen type **C17**. Another possibility is that the American and South African species that have the similar pollen types (**C17** and dC5) are not related but developed similar pollen types in response to similar selective pressures (convergent evolution).

5 CONCLUSIONS

- Pollen type dC subtypes dC1, dC2, dC3, dC4, dC6, dC7, dC9, dC10, dC11, **C16** and **C17** were recorded in the present study. Of these, pollen subtype dC6 is the most complex subtype observed in the present study. All these subtypes, except for **C16** and **C17,** were also recorded among South African members of *Oxalis* (Dreyer 1996). Subtype **C17** is very similar to the monotypic and complex pollen subtype dC5 of the South African species. The similarity between **C17** and dC5 could be as a result of convergent evolution.

- The pollen type dC (semitectate reticulate), which is most common among the South African species of *Oxalis,* was also recorded from 49 of the 50 American species included in this study. If common is primitive, then these results support Dreyer 's (1996) view that this is the most ancestral pollen type within *Oxalis.*

- The American sections *Thamnoxys*, *Holophyllum*, *Monoxalis* and *Heterophyllum,* which display various primitive morphological characters, also have the dC pollen type. This supports the idea of Dreyer (1996) that reticulate (dC type) pollen is primitive, and Huynh's (1969 a & b) suggestions that these sections may be ancestral to the South African taxa.

- More advanced pollen types and subtypes are observed from the south African members of *Oxalis* than from the American members. This strengthens the view that the South African *Oxalis* species are more derived than the American ones.

- Pollen type **E** is tectate with supratectal verrucae. It is the most advanced pollen type of those observed in the present study and was only recorded from a single species.

- Pollen types **C16**, **C17** and **E** in America and the monotypic pollen types in South Africa represent independent palynological evolutionary lineages within the genus.

- Grouping subtypes dC1, dC2, dC3, dC4 and **C16** seems to be taxonomically informative within the context of this study. No clear pattern could be observed regarding the relations among the subtypes dC7 – dC9.

- Subtypes dC10 and dC11 co-occur with pollen subtypes dC1 – dC4 and **C16**, dC7 and dC9 in sections *Laxae*, *Clematodes*, *Carnosae*, *Articulatae*, *Capillares* and *Alpinae*. Huynh (1969 a & b) already referred to shortcomings in the classification system proposed by Knuth (1930) and questioned the delimitations of sections *Laxae* and *Clematodes*. Further studies investigating taxonomic problems in these sections are necessary.

- The complex pollen subtype dC6 is regarded as monotypic in South African species of *Oxalis*. It also occurs in two American species belonging to different sections (*Ortigesae* and *Clematodes*). This may be as a result of convergent evolution, where the two species developed the same pollen type due to similar selective pressures. However, it makes more sense to think complex characters, especially of the pollen grain (because of its key role in reproduction), developed once in evolution rather than twice. An in depth study of these two species could reveal a closer taxonomic relationship than is currently recognized. Hence further investigation of these species is recommended.

- Pollen subtype **C17**, recorded from three American species, is very similar to the monotypic pollen subtype dC5 in South Africa. Pollen subtype dC5 is covered with supratectal spinules, while **C17** is not. Therefore dC5 can be regarded as more complex than **C17**. Pollen type dC5 may have been derived from **C17** in South Africa. It is also possible that the taxa in the two centres of diversity, with pollen types **C17** and dC5, exhibit convergent evolution of their pollen types.

GLOSSARY

Acetolysis - A technique for preparing pollen and spore exines for study. It involves acid hydrolysis to remove cellulose from pollen and spores leaving the exine.

Apertures – A region of the sporoderm that is thinner than the remainder of the sporoderm and generally differs in ornamentation and / or structure.

Areola – A feature of ornamentation in which the ectexine / sexine is composed of circular or polygonal areas separated by grooves which form a negative reticulum.

Baculum – A cylindrical, free standing exine element more than 1 μm in length and less than 1 μm in diameter.

Colpate – A pollen grain that has an elongated aperture with a length / breadth ratio greater than 2.

Colporate – A pollen grain that has a compound aperture with a colpus and porus combined in the same aperture.

Endexine – The inner part of the exine, which remains relatively unstained with basic fuchsin in optical microscopy and has a lower electron density in conventionally prepared TEM sections.

Equatorial axis – A straight line equivalent in length to the diameter of the grain at its equator.

Exine – The outer layer of the wall of a palynomorph, which is highly resistant to strong acids and bases, and is composed primarily of sporopollenin.

Footlayer – The inner layer of the ectexine.

Harmomegathic function of the pollen wall - The expansion and contraction of pollen wall during changes in hydration of pollen.

Hetromorphism - Different number of apertures in pollen grains of the same species.

Infra – A prefix for below or beneath, used for patterns underneath a complete or partial tectum, for example infrareticulate.

Intine – The innermost of the major layers of the pollen grain wall underlying the exine and bordering the surface of the cytoplasm.

Lumen (pl. lumina) – The space enclosed by the muri.

Mesocolpium – The area of a pollen grain surface delimited by lines between the apices of adjacent colpi or the margins of adjacent pores.

Murus (pl. muri) – A ridge or wall separating two lumina of reticulate, striate or rugulate sculpture.

Oblate - Description of the shape of a pollen grain or spore in which the polar axis is shorter than the equatorial diameter.

Panto- - A prefix for global distribution.

Pluricolumellate – The collumellae arranged in several rows beneath each murus.

Polar axis – A straight line between the distal and proximal poles of a pollen grain or spore.

Porate – A pollen grain that has a circular or elliptic aperture with a length / breadth ratio less than 2.

Prolate – Description of the shape of a pollen grain or spore in which the polar axis is larger than the equatorial diameter.

Punctum – A rounded or elongatetectal perforation, less than 1 μm in length or diameter.

Reticulum – A network-like pattern consisting of lumina or other spaces wider than 1 μm bordered by elements narrower than the lumina.

Rugulate – Describing a type of ornamentation consisting of elongated sexine elements more than 1 μm long, arranged in an irregular pattern that is intermediate between syriate and reticulate.

Semitectum – A partially discontinuous tectum in which the tectal perforations are equal to or wider than the muri and usually larger than 1 μm in diameter.

Sexine – The outer sculptured layer of the exine, which lies above the nexine.

Spherical – Describing the shape of a pollen grain or spore in which the polar axis and the equatorial diameter are approximately equal.

Spine – A word applied in palynology to long and tapering pointed elements, exceeding 1 μm.

Striate – elongated generally parallel elements separated by grooves.

Supra – A prefix for above, used mostly for features on top of the tectum in palynology, for example suprareticulate.

Tectum – The layer of sexine, which forms a roof over collumellae, granules or other infratectal elements.

Verruca – A wart-like sexine element, more than 1 μm wide, that is broader than it is high and is not constricted at the base.

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